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4613

Principal Inorganic Constituents of Diseased Cardiac Muscle.

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Analyses have been made on 57 hearts. The muscle segment for analysis was taken from the left ventricle except in the case where the heart was too small. Unfortunately fresh material was not available in the great majority of instances, but was obtained only from hearts that had been preserved in formalin for varying periods of time. Determinations of moisture, ash, P_2O_5, Fe_2O_3, CaO, MgO, K_2O, and Na_2O were made on muscle dried to relatively constant weight at 100° C. The clinical and anatomical diagnosis, as well as the age, sex and color was obtained in every instance and the usual run of cardiac maladies together with complicating diseases is well represented.

The determinations show wide variations between maxima and minima. The highest moisture percentage was found to be 83.23%, the lowest 69.94%. Ash varied between 4.666% and 1.016%; P_2O_5 between 2.130% and 0.315%; Fe_2O_3 between 0.320% and 0.008%; MgO between 0.212% and 0.012%; CaO between 0.463% and 0.005%; K_2O between 0.904% and 0.065%, and Na_2O between 1.871% and 0.200%.

One would expect the CaO to be high in the aged and in those presenting sclerotic changes. That this is not always the case is shown by the fact that in an instance of a person 81 years old with generalized A.S. and coronary sclerosis the CaO was only 0.140%, while in another individual of 49 years with coronary sclerosis and an obviously degenerated heart muscle the CaO reached 0.463%. In general, persons dying of tuberculosis had hearts with relatively low calcium content. However, this is not invariably the rule, for one heart from a tuberculosis patient suffering also from luetic infection and aneurysm of the aorta showed 0.116% CaO.

There is also marked variability in the K_2O content. The highest percentage obtained was in the heart from a boy of 14 who died of acute nephritis with anasarca. The K_2O was 0.904% while the calcium was 0.032%. The same determinations on the heart of a child 1½ years old gave 0.285% potassium oxide and 0.027% calcium oxide.

With the large variety of diseases represented and the relatively small number of hearts analyzed an arrangement of the percentages in accordance with disease is hardly justified at present.

So far it has been impossible to draw any definite conclusions, and while there is no correlation between any series of the constituents determined and the ages, there appears to be a tendency to grouping, within fairly narrow limits. It is clear that with so many variables, only a large number of analyses will yield results which will permit conclusions being drawn. Nevertheless there is some indication that a variation in both the calcium and potassium content of heart muscle at different ages as well as in different diseases exists.

Fresh hearts are now obtained with increasing ease, and the analytic method has been greatly improved while the errors incident to the numerous manipulations have been reduced or eliminated. It is hoped that with a larger series of analyses of normal as well as pathological hearts some relation between the alkaline earth and the alkali metals to age and disease, which is the object of this work, will become evident.

4614

Studies in Acetylphenylhydrazine Anemia in Dogs.*

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Anemia was produced in 5 adult dogs by subcutaneous injection of acetylphenylhydrazine (pyrodin). The amounts injected were calculated in terms of red cell volume as determined from hematocrit and plasma volume readings and varied from 40 mg. to 100 mg. per 100 cc. of red cells, given in single or divided doses. The dogs were fed a standard diet in order to minimize possible dietary effects.

Some degree of anemia, coincident with an increase in the icteric index, was present in all cases at the end of 24 hours. The anemia becomes most marked in 5 to 9 days after the first injection, the red cell and Hb determinations at this time showing values of from 33 to 67% of the original levels. Single injections produce a similar degree but a more rapid onset of the anemia than do divided doses of the same amount. The disappearance of the anemia is gradual, recovery beginning 12 to 16 days after the first injection. Approximately normal levels are reached in 3 to 5 weeks after injection. The percentage of reticulocytes increases to approximately double the normal values in 2 or 3 days after the pyrodin is injected and usually remains at this level. The first indication of recovery from the anemia is usually preceded by a further, sudden marked rise, the value reaching 33% in one experiment. The corpuscular volume shows a tendency to increase and to remain high even after the anemia has disappeared. The same is true of the resistance of the red cells which increases gradually and is greatest at the height of the anemia. The behavior of the white cells is irregular. The total is increased from 50 to 100% at the height of the anemia. No consistent changes occur in the differential or in the number of platelets. The Van den Bergh reaction was negative in all cases as was spectroscopic examination for methemoglobin.

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* Aided by a grant from the Committee on Radiation of the Division of Biology and Agriculture of the National Research Council.

4615

Evidence Against the "Latency Theory" of Partial A-V Block.

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There are two fundamentally opposing explanations of the characteristic groupings of beats separated by a pause when occasionally the ventricle fails to follow the auricle in low grade heart block (Wenckebach's periods). The "latency theory", Mobitz,[1] holds that the velocity of conduction through the muscular elements is invariable with constant auricular rate, and that the gradual prolongation of A-V interval following the pause in partial block is due to the arrival of the sinus impulse at the A-V junction at progressively earlier times during the recovery in the excitability on the ventricular side of the junction. Thus the impulse is regarded as arriving at the junction, pausing a moment, and then going forward. The duration of the pause is supposed to depend upon the excitability of the tissue immediately beyond the boundary plane.

That such a conception is inadequate is regarded as certain by some, and yet it is difficult to secure direct evidence against it.

The first evidence herein outlined is obtained from excised turtle hearts, rendered quiescent by removal of the sinus and driven at varying rates by break induction shocks applied to an auricle. In one experiment, after a 10 second rest, the auricle was twice stimulated, the interval between auricular responses measuring 3.026 seconds. The first A-V conduction time was 0.526 second; the second, 0.538. A 3 second rest was, therefore, almost sufficient for complete recovery of conductivity. In a second test after a 10 second rest the interval between the 2 transmitted auricular impulses was again practically 3 seconds, but an auricular response which was not transmitted to the ventricle was interpolated between the two, 1.156 second before the following transmitted auricular response. Although the ventricle did not respond to the interpolated impulse, the conduction time for the next impulse increased from the expected 0.540 second to 0.652 second. In a third test the interpolated response was elicited slightly later after the first, and was now transmitted to the ventricle in 0.614 second. The third auricular impulse, again 1.156 second later, reached the ventricle in 0.676 second.

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[1] Mobitz, W., *Z. f. Klin. Med.*, 1928, cvii, 449.

In the second test the *blocked* impulse had almost as great an effect upon the conduction time of the following impulse as did the *transmitted* impulse in the third test, although in the second test the ventricle was allowed a much greater time to recover from its refractory state. Obviously, under the conditions of this experiment, ventricular "latency" can play little or no part in prolonging the conduction time. This experiment is typical of the results in a considerable series of hearts.

The prolongation of conduction time observed in certain human cases of heart block is, as Mobitz recognized, much too long to be readily explained by a latency in the ordinary physiological sense. To surmount this difficulty he suggested that the "latency" in partial block is more complex, mediated in some fashion by the nerve cells concentrated at the A-V boundary; and he asserts that where such nerve elements are lacking, as in a ventricular strip, Wenckebach's periods are not obtainable.

However, it is possible to obtain true Wenckebach's periods by producing a region of block of the bridge type in transverse ventricular strips and by stimulating at the proper rate. In one such experiment the conduction intervals in seconds between the responses of the 2 ends of the strip, given in chronological sequence, were 0.29, 0.33, 0.39, blocked, 0.316, 0.40, 0.446, blocked. Mobitz's idea of the function of the nervous elements thus becomes gratuitous.

Examination of over 4000 human electrocardiograms has failed to reveal any cases not explicable on the basis of the more generally held conception, namely, that the variations in P-R intervals, either in partial A-V block or in other conditions, is due to a variation in the time of conduction from element to element of the conducting system, and not to a single exaggerated pause at one boundary.

4616

An Experimental Study of Hensen's Node in the Chick Embryo.*

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In making chorio-allantoic transplantations of the entire area pellucida of chick blastoderms in the head-process stage, Willier found that only head structures and heart developed in the grafts and the more posterior trunk structures failed to appear.[1] Since the only differentiation was of parts lying anterior to Hensen's node, the suggestion was made that the node may normally have a rôle in the formation of trunk structures at this stage but for some unknown reason did not realize its capacities in the grafts.

It was then suggested by Dr. B. H. Willier that I make an experimental analysis of the rôle which Hensen's node has in the development of the embryo. The problem was attacked by isolating and transplanting to the chorio-allantoic membrane transverse levels of the area pellucida at different stages of development as follows: (1) the node and other levels of the area pellucida of blastoderms in a stage prior to the appearance of the head-process, (2) the node and other levels of blastoderms in the advanced head-process stage and (3) the node level of embryos ranging from one to 12 somite stages.

The grafts obtained from the transverse levels of the area pellucida of blastoderms, in which a head-process was not yet present, showed that the node level has a great capacity for differentiation and is essentially totipotent. The structures which develop include all levels of the brain, neural cord with ganglia and nerves, epiphysis, eye, notochord, heart, gut and liver, cartilages with attached muscles, integument with feather germs, and mesonephros. Other levels of the area pellucida had very little capacity for differentiation. The transverse level anterior to the node and the streak posterior to the node showed only a development of gut, cartilage, muscle and integument.

Grafts of the node level from blastoderms with a head-process and beginning head fold showed a development of hind brain, neural cord, ganglia and nerves, notochord, heart, gut and liver, cartilages with attached muscles, mesonephros, integument with feather germs, and adrenal gland. There is thus a diminishing capacity of the node for differentiation when compared with the node of the earlier stage since the more anterior structures such as fore brain, eye, epiphysis and hypophysis do not appear. However, these structures and others develop from the level anterior to the node which indicates that there has been a determination of some of the anterior parts. The primitive streak posterior to the node has no greater capacity for differentiation than previously. However, if the posterior part of the node is included with it, additional structures such as neural cord, mesonephros and adrenal gland develop.

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* This work was done at the University of Chicago under the direction of Professor B. H. Willier.

[1] Willier, B. H., *Anat. Rec.*, 1926, xxxiv, 158.

Grafts from the node level of blastoderms having from one to 12 somites show that there is a further reduction in the capacity of the node. However, at all stages considered there develops neural cord with ganglia and nerves, notochord, cartilage and muscle, integument with feather germs; mesonephros, adrenal gland and gut.

The totipotency which the node exhibits, *i. e.,* the capacity to differentiate structures which represent different levels of the embryo, indicates that the node is a center of differentiation in the normally developing embryo. That it continues to be such a center in the later formation of the trunk may be seen by its continued although diminishing capacity for differentiation.

Since at all stages considered the node has the capacity to differentiate such structures as the neural tube and notochord, and since the primitive streak posterior to the node lacks this capacity, it is suggested that the primary rôle of the node is to form these axial structures. The factors which control the development of these structures have not yet been completely worked out but the preceding experiments would seem to indicate a definite correlation between the development of the central nervous system and the head-process. These experiments show that in stages just prior to the appearance of the head-process, the nervous system does not develop in grafts of either the level anterior or posterior to the node in spite of the fact that brain and neural cord form in these respective levels at a later period of development (Kopsch).[2] However, if the head-process is present or a part of the node is included in the grafted part, then the nervous system does develop from either level. A further indication of correlation is seen in the successfully grown grafts of the node from blastoderms of the various stages used. In 75% of such cases the notochord which develops from the head-process is observed to be in close proximity to the neural tube. It is consequently suggested that the head-process may be the first structure to differentiate and subsequently acts as an inducing agent for the differentiation of the nervous system both in the grafts and in the normally developing embryo.

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[2] Kopsch, F., *Z. f. Mikr. Anat. Forsch.,* 1927, viii, 512.

4617

An Inquiry into the Cause of Congenital Absence of the Gall Bladder.

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Out of some 2700 cats examined during the last few years, the writer has noted 3 kittens (2 females and one male) in which no trace of the gall bladder could be found. In one of these there was a large empty *fossa vesicae felleae* suggesting that perhaps the gall bladder had once occupied it and then been absorbed. In the other 2 there was neither fossa nor any fibrous remainder to indicate that the gall bladder had ever been formed; so that we may assume that it either failed to develop in the embryo or had disappeared before birth. According to Golob,[1] known instances of a congenitally absent gall bladder in man do not exceed 40. In some species of animals, as in the pigeon, a biliary vesicle is formed embryonically but its duct subsequently atrophies, leaving a detached bladder that soon degenerates; in others, as the rat, it is doubtful whether any anlage ever forms (Scammon[2]). But in rats, according to McMaster,[3] the loss of the biliary reservoir is compensated for by an increased concentration of bile, the bilirubin concentration being 8 times that in the hepatic ducts of the mouse—an animal having a gall bladder. In this connection it is interesting to note that one of the 3 cases of congenital absence of the gall bladder in cats was found in a kitten which was being operated upon. When a sample of the hepatic bile from this animal was examined with a colorimeter it was seen to be 2.2 more concentrated than a sample of hepatic bile from a control kitten, suggesting that if the gall bladder is lost early enough in fetal life, the deficiency is partly overcome by a compensatory mechanism.

In the hope of finding a possible explanation for this anomaly the writer examined serial sections of some 200 cat embryos of appropriate age in the Huntington Collection of Columbia University, and to his great surprise found a gall bladder in a 10.5 mm. embryo which was in the very act of disappearing. Indeed, the cystic duct had already atrophied, leaving the terminal vesicle isolated in the ventral body wall at a point just medial to the place where the right umbilical vein enters the liver. From this early embryonic position the gall bladder normally recedes. But in this particular embryo, withdrawal of the gall bladder had apparently been prevented by a circlet of veins which enclosed the neck of the organ, as a result of which the cystic duct would seem to have first thinned out and then ruptured. Two of these veins were of unusual interest since they exhibited an anomalous forking of the central end of the vitelline vein; so that instead of following its usual circuitous course to the liver through the portal mesentery, this vein had bifurcated, one limb of it anastomosing with the adjacent right umbilical vein and the other extending straight through the *septum transversum* to that portion of the left common cardinal vein which is destined to become the coronary sinus. Whether the actual rupture of the cystic duct was due to the pressure of these anomalous veins is, of course, a matter of conjecture. But at least it shows the very early period at which the gall bladder disappears and indicates that the method is not unlike that which normally occurs in the pigeon, namely, isolation and atrophy following rupture of the cystic duct.

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[1] Golob, Meyer, *J. Am. Med. Assn.*, 1927, lxxxix, 691.

[2] Scammon, R. E., *Anat. Rec.*, 1916, x, 543.

[3] McMaster, P. D., *J. Exp. Med.*, 1922, xxxv, 127.

4618

Observations Upon the Nature of the Virus of Hog Cholera.

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Although hog cholera has been the subject of extensive investigation by immunological methods, little has appeared in the literature concerning the nature of the specific virus, aside from the discovery of DeSchweinitz and Dorset[1] that the causal agent is filtrable through Berkefeldt and Chamberlain candles. This paper records certain observations made with the virus in the course of an experimental study.

The virus in the blood of hog cholera withstands rapid desiccation over sulphuric acid, unslacked lime or other hygroscopic agents *in vacuo* at 0° C., without its infectiousness and antigenic property being apparently affected. The resulting virus-powder may be kept in sealed glass containers for years and probably indefinitely without loss in viability, virulence and antigenicity. Though the virus in the powdered form keeps indefinitely, after it is redissolved in a suitable solvent such as normal physiological salt solution, it loses in viability and antigenic property at the same rate and under like conditions as is the case with the undesiccated virus-blood.

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[1] DeSchweinitz and Dorset, Cir. No. 41, Bureau of Animal Industry.

The desiccated virus-serum can be utilized in conjunction with immune serum (desiccated or fresh) to produce an active immunity in the hog. The virus-powder is first thoroughly dissolved in the fresh immune serum or in the dissolved desiccated immune serum in the proportion of one part virus to 2 parts serum. This *in vitro* admixture of virus and immune sera in no way prevents the production of an active immunization of the host following its subcutaneous injection, and gives rise to no objective symptoms of infection. The virus-powder and desiccated immune serum may be mixed and kept for an indefinite period in a sealed glass container, away from light and at 0° C., without appreciably affecting its antigenic power. These facts permit of the deduction that the virus is not destroyed *in vitro* through the action of a lytic immune body, nor is it apparently affected *in vivo* through the additional action of host complement. Repeated experiments with activated and inactivated virus-immune serum mixtures show conclusively that the immune body in hog cholera is antitoxic rather than lytic. Viability and virulence of the virus-powder are not affected when mixed with fresh immune serum. The mixture in which the immune serum is less in quantity than the virus, per mg. of weight, the animal injected invariably develops cholera. A larger quantity of immune serum than virus-serum is employed in making a mixture sufficiently potent to neutralize the toxin elaborated during the period in which the animal is acquiring an active immunity. That the virus is viable and multiplying in the injected hog is confirmed by the fact that the withdrawn blood is highly infectious.

It has been determined by animal experiment that the virus of hog cholera at no period during the infection is intracellular, in so far as the blood cells are concerned. Repeated injections into young non-immune hogs of large quantities of the freshly washed blood cells have failed to infect. Therefore, it is highly advantageous in working with the virus to eliminate the cellular elements of the blood and use only the serum since the cells are worthless from the standpoint of virus. To obtain the cell-free virus-serum the freshly drawn blood of the infected hog is first defibrinated and then centrifugalized under sterile conditions, and the clear supernatant serum pipetted off.

By the method of desiccation, 1 cc. of virus-serum yields approximately 100 mg.; the dried defibrinated whole blood gives a residue of 156 mg.

While the virus content of infected serum varies depending upon a number of factors, as the size and age of the hog, duration and severity of the infection, commonly 1 cc. of cell-free virus-serum contains approximately 4000 fatal doses for the hog of 40 to 50 pounds weight. Expressed in terms of desiccated virus-serum the minimum lethal dose is 0.01 mg.

Our attempts to cultivate the hog cholera virus upon artificial media have met with only partial success. No growth of the virus occurs on ordinary laboratory media under any conditions. Excepting where semi-solid hog plasma medium was employed, prepared after the method of Noguchi,[2] the inoculated virus rarely survived longer than 3 weeks regardless of the temperature at which the cultures were maintained. Multiplication apparently takes place in the special hog plasma medium when kept under anaerobic conditions and at 40° C. While colonization of the virus was at no time observed in the plasma medium there was noted in the deeper parts a cloudiness similar to that produced by the artificially grown *Treponema pallidum*. Further proof that virus growth had occurred was shown by infection of the hog with the sixth subculture in amounts no greater than the original material used for culturing. It is unlikely that the virus simply remained viable and was carried over to subcultures in sufficient numbers to infect. Preparations made from the cultures and examined with the dark field show only innumerable minute globular bodies which are similar in morphology to those described by Flexner and Noguchi[3] for poliomyelitis. Nothing whatsoever was seen in the cultures that even resembled spirochaetal organisms. It is of interest to note that frequently *B. cholera suis* (T. Smith) grew out in pure culture from the desiccated virus-serum. In one instance a pure growth of the hog cholera bacillus was obtained from virus-powder more than 2 years old.

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[2] Noguchi, H., *J. Exp. Med.*, 1919, xxx, 13.

[3] Flexner, S., and Noguchi, H., *J. Exp. Med.*, 1913, xviii, 461.

4619

Cultivation of Entamoeba Histolytica From a Hepatic Amoebic Abscess.

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Through the employment of Locke egg-serum medium for the culture of *Entamoeba histolytica* as used by Boeck and Drbohlav[1] and somewhat augmented by Dobell and Laidlaw,[2] the cultivation of this protozoa has now become of very practical service. Nearly all such isolations have been recovered from the intestinal tract of man. In many of the reported instances there exists considerable doubt as to the species of the cultivated amoeba. There is no question, however, that such microorganisms of the histolytica type have been cultivated, as many of the reports are by workers who are recognized authorities in this field and therefore well versed in the biological and morphological features recognized as differential and diagnostic for such species. Dobell[3] reports the cultivation of amoeba from different types of monkeys which he indicates are not to be differentiated from *Entamoeba histolytica* of man. This is of much importance in the consideration of the experimental transmission of this infection to such types of anthropoid animals.

The opportunity was recently presented of investigating a very typical instance of amoebic dysentery in man with a complicating amoebic abscess of the liver. At operation a specimen of pus-like material was removed from the hepatic lesion. Cultures and smear preparations of this specimen were made for ordinary bacteria and found negative. The subject died 24 hours after the operation and at autopsy the liver showed extensive destruction or so-called abscess formation. Examination of the fresh material scraped from the necrotic walls revealed many actively motile amoeba. Cultures of such material were prepared in Dobell's modification of the Boeck-Drbohlav medium and the amoeba grew freely in certain of the tubes. The culture has been carried for 5 months representing approximately 30 generations.

While the smears of material obtained at the time of operation as well as the cultures made on routine laboratory media showed no contaminating or pathogenic bacteria, the primary amoebic cultures made post-mortem from the scrapings of the abscess wall showed definite contamination composed in greater part of members of the colon group. It is apparent that bacterial invasion had occurred post-mortem during the interval between death and the autopsy, which was approximately 12 hours.

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[1] Boeck, W. C., and Drbohlav, J., *Am. J. Hyg.*, 1925, v, 371.

[2] Dobell, C., and Laidlaw, P. P., *Parasitol.*, 1926, xviii, 283.

[3] Dobell, C., *Parasitol.*, 1927, xix, 288.

The examination of the material from the scrapings of the liver wall showed many actively motile amoeba, the general features of which corresponded to those of the histolytica type. Smear preparations were made from this material and from the cultures and were stained by Heidenhain's iron haematoxylin method. A study of these stained specimens revealed the morphological features that have been regarded as indicative of the species histolytica.

The microscopic study of sections of the liver showed the characteristic autolysis of the hepatic structure as evidenced by massive necrosis with karyorhexis and karyolysis and few, if any, inflammatory cellular elements were present. Many amoeba were present in the tissue sections and were found especially at the margin of the necrotic and partially injured liver tissue..

While cultures from the intestinal tract may present a varied assortment of non-pathogenic amoeba, there seems but very remote likelihood of such condition occurring in the liver lesion wherein a definite clinical and pathological picture of amoebic infection exists, as was seen in the present instance.

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Injection into the Rabbit of Cultures of Entamoeba Histolytica.

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Cultures of *Entamoeba histolytica* obtained from the stools of human cases of amoebic infection have been introduced into the intestinal tract of kittens by Cutler[1] and Boeck and Drbohlav[2] and the disease apparently successfully reproduced in this type of animal.

A rather generalized systemic invasion by *Entamoeba histolytica* has also been described by other workers, but there are many who question the cellular structures considered as amoeba in such reports. The invasion, however, of these protozoa into the liver with the resulting autolysis of hepatic tissue and the formation of the so-called amoebic "abscess" has been generally accepted as occurring through the portal system. The study of the histopathologic lesion in the liver often reveals numerous amoebas in the vascular pathways, suggesting this system as the route of invasion.

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[1] Cutler, D. W., *J. Path. and Bact.*, 1918, xxii, 22.

[2] Boeck, W. C., and Drbohlav, J., *Am. J. Hyg.*, 1925, v, 371.

In the present experiments, having obtained at autopsy a satisfactory culture of *Entamoeba histolytica* from a hepatic "abscess," as herein reported[3] we were desirous of ascertaining the effects that might be produced by hypodermic injections of such a culture into the lower animal through various routes of introduction.

The culture contained a varied bacterial flora in conjunction with the luxuriant growth of the amoeba. The presence of the bacterial microorganisms in the injection material naturally somewhat confuses the results.

Twenty-five rabbits were employed.

Series 1.—Eight rabbits were laparotomized and fresh cultures rich in amoeba were introduced with a hypodermic syringe into the branches of the mesenteric veins. Such suspensions were naturally carried through the portal system and into the liver. Five of these animals were killed at varying periods of from 2 to 5 weeks. At autopsy evidences of localized peritonitis were present about the site of inoculation but resolution and organization of exudate were shown in such areas. The remainder of the animals died of generalized peritonitis in from 24 to 72 hours. In these, some of the inoculation material had dropped into the cavity at the time of the injection. The livers of all 8 rabbits were sectioned serially and no evidences of gross changes were found. Smear preparations were made from the cut surfaces and while a few bodies suggestive of cysts were seen no definitely motile amoeba were present. It is remarkable that the presence of the inoculum in the peritoneal cavity provoked at all times a reaction and in some instances caused death, whereas the bulk of such material introduced intravenously produced little if any response when carried to the liver by the portal system. This serves as additional evidence of the defensive mechanism of the liver structure to invading microorganisms.

Series 2.—Seven rabbits were injected directly into the liver substances. In 2 of these, the needle was introduced from without the body into the liver and in the other 5, the animal was laparotomized and the needle introduced directly into the liver structure. One of the animals injected through the wall died after 3 days and a peritonitis was found in the upper portion of the peritoneal cavity. The remaining 6 were killed after 2 to 6 weeks and again evidences of local reaction due to the injected material were found over the surface of the liver about the point of inoculation. Sections of these livers revealed no localized abscess formations, and smears and cultures for amoeba were negative.

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[3] Friedrichs, A. V., and Harris, W. H., PROC. SOC. EXP. BIOL. AND MED., in press.

Series 3.—Four rabbits were inoculated into the marginal ear vein with a culture suspension containing numerous amoebae and the accompanying microorganisms. Two of these died in 3 to 6 hours and 2 survived and were eventually killed. Smear preparations were made from the lungs, heart, liver and spleen of all animals and in no instance could the amoeba be found.

Warthin[4] states that spermatozoa were found within the amoeba in human testes. It was thought therefore that in this organ lesions might be formed in which the amoeba would flourish *in vivo*.

Series 4.—Six rabbits were inoculated into the testis with an amoebic culture suspension. A very marked acute inflammatory reaction followed, due, no doubt, to the bacteria present in the suspension. Considerable local tumefaction occurred for all animals and 2 died in 10 days and 2 weeks respectively from a septicemia. Three died within 2 months and one still survives 4 months after the inoculation and presents a marked tumefaction of the testis and scrotum. Specimens of fluid were aspirated from the inflammatory masses either with a hypodermic syringe or a glass capillary pipette with rubber teat attached. These examinations have been made frequently and at repeated intervals but in no instance were actively motile amoeba found. At certain times when encysted amoeba were found cultures were prepared but no growth of amoeba was obtained.

Although freshly isolated cultures rich in *Entamoeba histolytica* were introduced directly into the liver tissue and also into this organ through the portal system, in no instance did this parasite produce a lesion. It is possible that the bacteria present provoked a transitory response on the part of the tissues causing destruction of the amoeba. It might also be a fact that the animal chosen, namely the rabbit, is not susceptible to this infection.

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[4] Warthin, A. S., *J. Infect. Dis.*, 1922, xxx, 559.

4621

The Life Cycle of the Rare Human Tapeworm, Taenia Confusa Ward, 1896.

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In March, 1929, there was referred to the writer for diagnosis a complete strobila of an unarmed tapeworm, passed by a native white woman from St. John Parish, Louisiana, after administration of oleoresin of male fern. The worm grossly resembled *Taenia saginata,* but differed in several specific characters, including a more distinctly oval scolex, a pair of lateral elytra along the unsegmented region behind the scolex, longer and narrower terminal proglottids, and more clumped, less discrete branches of the uterus in these proglottids. In view of the fact that only 3 cases of this infection were on record (Nebraska, 2, Ward,[1] Guyer,[2] Texas, 1, Chandler[3]) and that this specimen was the first one to be diagnosed in the unpreserved condition, it seemed desirable to attempt to determine by experimental feeding what animal served as larval host. Information elicited from the patient indicated that she occasionally ate pork, rabbits and deer meat, and that she was fond of raw beef.

A 2-weeks old suckling calf and a rabbit were therefore secured and were each fed macerated terminal proglottids (*e. g.,* gravid segments) of the tapeworm. After a period of 3 months, during which time the animals were kept under rigid supervision, they were autopsied. The rabbit was found to contain 3 specimens of an armed cysticercus, *C. pisiformis,* a natural infection of rabbits, while the calf was infested with more than a thousand cysticerci, which had unarmed scolices, and which were considerably smaller than those of *Taenia saginata.* The extremely heavy infestation of the calf under circumstances that practically precluded a natural infection with larvae of *T. saginata,* together with the smaller size of the cysticerci recovered, has practically established the fact that these larvae were the resultant growth from the eggs which had been fed 3 months previously. Evidence therefore favors the view that the ox is an acceptable larval host and probably the normal one of *Taenia confusa,* and that the rabbit is not an appropriate larval host of this worm.

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[1] Ward, H. B., *Western Med. Rev.,* 1896, i, 35-36.

[2] Guyer, M. F., *Zool. Jahrb.,* 1898, xi, 469.

[3] Chandler, A. C., *J. Parasitol.,* 1920, vii, 34.

Examination of these cysticerci proved that they were fully mature. Experimental human infection did not seem desirable or necessary, but several of the larva were fed to each of 2 laboratory monkeys. More than 3 months later the monkeys were still negative. There is therefore no evidence that any primate other than man can serve as definite host of this tapeworm.

The experimental proof of the life cycle of *Taenia confusa* directs attention to the undoubted existence of this species of tapeworm in the Southern and Southwestern United States, both in the adult state as a human parasite and in the larval stage in cattle.

4622

Effects of Bacteriophage Upon Cultures of Entamoeba Histolytica.*

FRANK GEMAR. (Introduced by W. H. Harris.)

From the Department of Pathology, College of Medicine, Tulane University and the Laboratory of the Veteran's Hospital 84.

The cultivation of *Entamoeba histolytica* has been made of practical and almost routine laboratory usefulness through the Boeck and Drbohlav[1] method of procuring cultures and the modification of Dobell and Laidlaw[2] has enhanced its success. However, the maintenance of continuous generations is often impaired and the strains frequently lost through the concurrent bacterial growth. As Dobell and Laidlaw have pointed out, the conjoint protozoal and schizomycetic microorganisms may attain an adaptation or adjustment that makes the amoebic growth luxuriant and the propagation of the culture simple. However, in continuing strains through many generations, the contamination or bacterial overgrowth feature is a most annoying factor and often threatens to destroy or actually causes the loss of the Entamoeba culture. As far as known the cultures of amoeba obtained by this method are always accompanied by bacterial growth and these accompanying microorganisms are regarded by many as obligatory commensals or symbionts to the protozoal culture. Many efforts have been made, however, to reduce the bacterial contamination both as regards numbers and species. Since nearly all cultures have been obtained from the intestinal lesions, the bacterial accompaniment is naturally extensive.

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* I wish to extend my thanks to Dr. W. H. Harris for his assistance and the suggestion of the problem.

[1] Boeck, W. C., and Drbohlav, J., *Am. J. Hyg.*, 1925, v, 371.

[2] Dobell, C., and Laidlaw, P. P., *Parasitol.*, 1926, xviii, 283.

Dobell and Laidlaw's modification of cultivation includes a weak solution of Acriflavine for the purpose of inhibiting overgrowth of bacteria especially of the starch splitting variety. This chemical must, however, be used in very weak strengths as otherwise it will destroy the amoeba themselves. In our hands the bacterial growth was only slightly influenced by these weak solutions, although the rice starch was better preserved.

In the present experiments the employment of bacteriophage in conjunction with the cultivation of *Entamoeba histolytica* was carried out with the view to inhibiting the bacterial growth occurring in the supernatant fluid medium. The culture of amoeba was obtained by Harris and Friedrichs[3] from an amoebic abscess of the liver procured at autopsy. It was thought that this culture would contain bacteria consisting only of the colon group; it was, however, found that many Gram positive microorganisms were also present.

In the first experiments, a race of phage was used, obtained several years previously from Yale. As this material was of rather low titer and could not be exalted by repeated passage, it was later abandoned. A sample of sewage was then collected from the main sewer of the Veteran's Hospital and passed through a Seitz-Wertz filter. From this was procured a bacteriophage with a final potency of one to ten billion for strains of both *B. coli communior* and *communis*. While this phage was highly potent for the laboratory stock cultures employed, it acted only feebly upon the colon strains grown from the culture of Entamoeba.

Numerous experiments were carried out employing this colon phage in conjunction with the entamoeba cultures. The phage was introduced in varying amounts at the time of transplanting the amoebic cultures. As a whole the results indicated that definite inhibition of bacterial growth was often demonstrated for a short period of time, but this was only transitory. The recurrent bacterial growth showed masses of gram positive microorganisms as well as some gram negative bacilli. It was, therefore, found that no clear cut or consistent phagic action could be obtained with this particular bacterial flora.

An attempt was next made to supplant the microorganisms present in the supernatant fluid of the medium with the strains of *B. coli* upon which the phage employed had proven highly effective. Heavy suspensions of these colon strains were inoculated into the supernatant fluid at the time of transplanting the amoeba, hoping through this method to crowd out those present in the primary culture and thereby procure a flora vulnerable to the phage. Very little, if any, better results were obtained and apparently the original microorganisms could not be displaced.

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[3] Harris, W. H., and Friedrichs, A. V., PROC. SOC. EXP. BIOL. AND MED., in press.

The employment of bacteriophage appears desirable in reducing the bacterial growth present in amoebic cultures. While certain bacteria, especially *B. coli,* may be essential in the *in vitro* growth of *Entamoeba histolytica* it is likely that the phaged microorganisms may likewise supply the necessary food factor. Again if whole bodies of microorganisms are needed, killed cultures could be added. The only mitigating factor that is therefore to be considered, is whether or not the activities of the bacteria themselves are obligatory.

While it is true that for practical laboratory purposes the presence of a heavy bacterial growth in amoebic cultures is of no especial importance, from the experimental standpoint a pure culture of amoeba or one containing a minimum bacterial content is greatly to be desired. It is likely that such cultures will prove more easy to propagate.

Though varying amounts of a bacteriophage of considerable potency were introduced into the amoebic cultures, in no instance was evidence of interference with this protozoal growth manifested.

Pacific Coast Branch.

Stanford University, October 19, 1929.

4623

A Study of Certain General Conditions Leading to the Differentiation of Cells.

MONTROSE T. BURROWS.

From Private Research Laboratory, Pasadena, California.

In previous articles[1, 2] I have given evidence to show that heart muscle cells may show a variety of reactions and changes in the tissue culture. It was shown further that each of these various forms of reactions and changes is a response of the cell to a given environment.

Under certain conditions these cells may stretch out from the fragment as an otherwise inactive syncytium. Again they are seen in other cultures migrating out as large mononuclear cells. Under other conditions they separate, flatten on the surface of the medium, grow actively, divide by mitoses and resemble actively growing mesenchyme or sarcoma cells. In other cultures they migrate from the fragment as single spindle-shape, mesenchyme-like cells and they have been seen to differentiate into rhythmically contracting cells. These latter cells have been removed from the environment suitable for such activity to one suitable for growth and division, and have changed from contracting cells to cells which flatten on the surface of the medium, grow actively and divide by mitosis.

A careful study of the condition suitable for these various activities has shown that the growing cells are cells flattened against a film of material which flows out from the fragment over the surface of the medium. The syncytia which stretch out from the fragments and the migrating spindle shaped heart muscle cells are cells which migrate directly into the clotted plasmatic medium from fragments which do not disintegrate and throw out a surface film. The

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[1] Burrows, M. T., *Am. J. Physiol.*, 1917, xlv, 556.

[2] Burrows, M. T., and Johnston, C. S., *Archiv. Int. Med.*, 1925, xxxvi, 293.

large mononuclear cells are cells migrating into a clot of plasma which fails to coagulate to fibrin and serum but which is undergoing slow dissolution. The contracting cells are cells which are either stretched through the serum of the coagulated plasma clot between well formed fibrin fibrils and a surface film of material which has spread out from the fragment, or cells which are stretched through the serum between the fragment itself and the ends of fibrin fibrils.

Contraction in these cells does not begin at once but always late in the life of the culture, when the plasma clot in the neighborhood of the fragment has become transformed into dense fibrin fibrils and serum.

Cells placed in the fibrin of such cultures become immediately inactive. Cells placed in the serum round off, become inactive and show the sharp conture of no or very little interchange with the medium. In the surface film alone do these cells flatten out, show a low surface tension and evidence of activity. In the contracting cells we could believe, therefore, that activity was located only at the one end in contact with the surface film or the fragment. They are polarized cells. Contraction, we could believe, is the result of this polarization of the muscle cells.

The next question is whether this polarization is peculiar for inducing rhythmical contractions in cells or is it necessary for function in all cells? It had been suggested to me by several biologists that I would probably find that simple mesenchyme and other cells will contract rhythmically if polarized in a similar manner in the culture.

After applying this condition of polarization of the functioning heart muscle cell to the body as a whole we appreciated that probably all functioning cells of the body are polarized in a similar manner. The gland cells are stretched between a basment membrane, a rich net of capillaries, nerves, lymphatics, etc., and the open gland duct. The nerve fibers are stretched between the brain and an end organ. It is the nerve fibers which exhibit pain and not the brain itself.

It seemed possible, therefore, that the polarization noted in the contracting heart muscle cell is peculiar to all functioning cells and that the type of function is determined by the cell itself, differentiated from the egg as it is at a much earlier time. The fact that cancer may arise from any tissue suggests again that the conditions leading to a change from a state of function to a state of growth may be similar in all tissues and that the type of function may be a fixed property of the cell itself.

It became of interest to study the reaction of striated skeletal muscle, smooth muscle, simple mesenchyme and various forms of epithelial cells in the cultures. After preparing more than 30000 cultures of these tissues, I observed that each type under certain conditions can grow, can show migration without growth and also at times some evidence of that form of differentiation peculiar to them in the mature organism. Three isolated contracting skeletal muscle cells and 2 isolated contracting smooth muscle cells were observed. In each instance these contracting cells were polarized in a manner quite identical to the isolated contracting heart muscle cells. The skeletal muscle cells had a rhythm identical to the heart muscle cells. They contracted at a rate of about 90 per minute. The smooth cells contracted but they showed the slow rhythm peculiar to these cells in the body. A few polarized mesenchyme cells were also observed but they showed no evidence of contraction. Single polarized epithelial cells were never seen. These cells dissolve the fibrin clot very soon.[3] Their only evidence of differentiation is the formation of tubes or alveoli. Membranes of epithelial cells are formed readily in the cultures and they are invariably stretched between ends of fibrin fibrils and the fragment through a liquid cavity. Evidence of rhythmical contraction was never observed in such membranes. Nerves frequently become stretched also between the fragment and the fibrin through a serum cavity but they never show any evidence of contraction.

The snappy rhythm of the contracting heart is evidently a peculiarity of striated muscle cells in general in the body. The same conditions which produce this rhythm in these cells induces the characteristically slow rhythm in the smooth muscle cells and causes no contraction whatsoever in the simple mesenchyme cells and other cells.

Some experimental evidence has thus been obtained for the first time which indicates that the peculiarities of the function of the various cells of the body are a property of these cells differentiated from the egg at an earlier time. The change of the cell from its early growing embryonic phase to the differentiated and functioning adult phase and vice versa is due on the other hand to common general changes in the organization of the whole. Further evidence is also given to show that function instead of growth in these cells is due to external influences limiting activity to one end of these cells or, in other words, it is due to a polarization of the cell.

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[3] Burrows, M. T., *J. Cancer Res.*, 1927, xi, 72.

4624

Potent, Sterile and Low-Protein Extracts of the Growth Hormone From the Anterior Hypophysis.*

HERBERT M. EVANS, ROBERT E. CORNISH AND MIRIAM E. SIMPSON

From the Department of Anatomy, University of California.

The following procedure has been used in obtaining extracts of the growth hormone of the anterior hypophysis for producing experimental gigantism in rats and provoking the growth of hypophysectomized puppies in the hands of Dr. Frederick L. Reichert.

2,000 gm. of beef anterior hypophysis. The anterior lobes of beef hypophyses had been stored for a period of between 18 and 24 months with solid CO_2 in an insulated container in a room held at —5.0° F., after having been ground in a Wiley laboratory mill.

12,000 cc. distilled water.

3,000 cc. 0.2/N $Ba(OH)_2$

Centrifugation: The alkaline mass was mixed by an electric household stirrer ("kitchen mechanic"); then centrifuged in a Sharples laboratory centrifuge. Large nozzles were used in the first centrifuging in order to remove the bulk of the insoluble material; the extract was then centrifuged at a rate of about 10 cc. per minute. The centrifuge was cooled with salt and ice at all times and the extract was kept surrounded with ice.

Neutralization: 0.2/N H_2SO_4 was added slowly with constant stirring by the same stirrer. Phenol red was used as indicator and the end point chosen was about pH 8.0, about 500 cc. being required. The Ba not already precipitated by H_2SO_4 was removed by a slight excess of Na_2SO_4.

Centrifugation: Sharples centrifuging was then repeated, finishing as before at a rate of 10 cc. per minute.

Filtration: A standard 14 cm. Seitz asbestos filter was used. A pressure of about 10 lbs. per square inch was applied by the use of a tire pump. Time required for filtration of 4 liters was about 45 minutes; towards the end, the rate of flow through the filter had decreased to half the original rate. Precautions were taken to maintain sterility of the solution after filtration. The extract was stored in 25 cc. lots in rubber-capped bottles and kept frozen until needed for injection.

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* Aided by grants from the Committee for Research in Problems of Sex of the National Research Council and the Board of Research of the University of California.

Yield: Approximately 15 liters.

Temperature during preparation: It is important to note that the temperature of the extract was never allowed to rise above 10° C. at any time during the preparation.

Bacterial content: Culture of the extract proved it to be sterile. (Dr. Reichert.)

Total solids: About 1.1%.

Color: Very pale, cloudy pink, presumably due to hemoglobin, almost clear in 100 cc. bottles; fluorescent.

Potency: Tested in normal adult female rats (weighing 250-270 gm.) the extract gave what we consider to be indubitable proof of the presence of the growth hormone; it created an increase in body weight of about 35 gm. in 20 days when administered in a daily intraperitoneal dose of 1/8 cc. and a growth of 55 gm. when given in 1 cc. daily doses.

4625

Effect of Morphine Sulphate by Mouth on Oxygen Consumption in Normal Humans.

H. H. ANDERSON. (Introduced by C. D. Leake.)

From the Pharmacological Laboratory of the University of California Medical School, San Francisco.

A preliminary report by M. E. Stark[1] has shown that morphine sulphate, presumably administered subcutaneously, significantly decreases oxygen consumption in normal humans, and, as might be expected, in a manner roughly proportional to the dose (0.37 mgm. to 0.48 mgm. per kg. weight). In studying the effects of the commonly used barbiturates on oxygen consumption, observation was also made of the action of morphine sulphate on some of the subjects. The drug was administered by mouth in doses from 0.1 mgm. to 0.5 mgm. per kg. weight. The technique was essentially the same as that employed by Miss Stark, except that the experiments did not run as long. The Sanborn apparatus and averages were used.

The results are shown in Table I. Where a change occurred in respiratory rate, it was usually slightly diminished. On the other hand, within the first hour after giving the drug, the pulse rate in-

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[1] Stark, M. E., *Analgesia and Anesth.*, 1929, viii, 307.

TABLE I.
Maximum Functional Changes during One Hour's Observation Following Oral Administration of Morphine Sulphate in Normal Humans.

Subject	Sex	Age	Wgt. kg.	Dose mgm/kg.	Resp. per min.	Pulse per min.	Pulse Pressure mm. Hg.	Tactile Discrim.	Basal Metabolic Rate
D. C.	M	40	70.5	0.1	—2	0	—8	Decrease	0
H. P.	F	28	61.0	0.1	+3	—4	—8	No change	—5
R. M.	M	22	65.0	0.1	0	+6	+8	Decrease	0
D. C.	M	40	70.5	0.2	—3	—12	+4	Decrease	—15
H. P.	F	28	61.0	0.2	0	+12	+6	Decrease	—7
R. M.	M	22	65.0	0.2	0	+6	—10	—	+3
A. C.	F	23	60.0	0.25	0	+6	0	—	—11
H. P.	F	28	61.0	0.3	—2	+12	+8	Decrease	—3
A. H.	M	25	72.0	0.3	—2	+6	+12	Decrease	—8
H. P.	F	28	61.0	0.4	0	+8	+8	Decrease	—5
R. M.	M	22	65.0	0.4	0	+14	+14	Decrease	—4
R. M.	M	22	65.0	0.5	0	+6	—16	Decrease	—5

creased quite markedly. Pulse pressure usually increased during this period. Tactile discrimination was generally diminished, and, in the higher dosages, to a considerable extent. The basal metabolic rate was generally depressed but not to the same degree as noted by Miss Stark. This may be a reflection of the presumed difference of administration and also of the fact that our experiments did not run longer than an hour. In this time there seemed to be no significant relation between the effect on oxygen consumption and the dosage employed which was in the ordinary therapeutic range. It is probable that longer observation would show a diminution in the pulse rate below normal and a greater fall in the basal metabolic rate after the oral administration of morphine sulphate in the higher dosages here employed. None of the subjects here noted had any symptoms of nausea or sleepiness following the drug.

4626

Effect of Amytal Anesthesia on Glucose Tolerance.

J. M. D. OLMSTED AND GEORGE GIRAGOSSINTZ.

From the Division of Physiology, University of California, Berkeley, Calif.

That amytal may have an effect on carbohydrate metabolism is indicated by the finding that dogs under amytal anesthesia exhibit a decreased capacity to assimilate injected glucose.[1, 2] This problem

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[1] Hines, H. M., Boyd, J. D., and Leese, C. E., *Am. J. Physiol.*, 1926, lxxvi, 293.

[2] Hines, H. M., Leese, C. E., and Baker, A. P., PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 736.

can perhaps be more readily investigated by employing the "glucose tolerance test." In our experiments this consisted in administering 2 gm. Pfanstiehl glucose per kilo body weight dissolved in 50 cc. water, followed by 100 cc. water. The changes in arterial (femoral) blood sugar were then followed at half hour intervals for 3 hours. In unanesthetized dogs the blood sugar rose to a maximum of some 300 mgm. within about one hour, and at the end of 3 hours had fallen to about 180 mgm.

Out of 5 dogs under amytal which were given sugar by means of a stomach tube, 3 showed no rise in blood sugar, and the other 2 showed a rise of only 10 mgm. In our experiments amytal *per se* produced no change in blood sugar (*cf.* Page,[3] Weiss[4] and others). One of these dogs was killed at the close of the test and the pyloric sphincter was found tightly closed, all the sugar still remaining in the stomach.

The abdomen of another amytalized dog was opened and sugar injected by syringe directly into the stomach. Again there was no rise in blood sugar during the following 3 hours, and again the sphincter was seen to be tightly closed. The stomach was emptied, washed out, and sugar now injected directly into the duodenum. This time there was a distinct rise in blood sugar, but the maximum reached was less than 2/3 the maximum when the test was tried on this same dog unanesthetized, and the blood sugar fell to normal before the end of the 3 hours. This finding was fully confirmed in 18 tolerance tests taken on 5 different dogs, 8 unanesthetized and 10 under amytal, the sugar being introduced through a tube into the duodenum (*cf.* Fig. 1). No sugar was found in the urine of the dogs under amytal at the end of the test and in the stomach there was not more than 5 cc. of liquid, which in 2 cases proved to be free from sugar and in 2 others contained a trace only. Two dogs were killed at the end of the 3 hour period and, as in the other cases, the pyloric sphincter was found tightly closed, the stomach and intestines almost toneless and intestinal movements feeble. The oesophagus and rectum were tied off and the entire alimentary tract removed, washed externally, then slit open, the contents washed out, and the amount of reducing substances estimated. In one dog this was equivalent to 5.28 gm. of sugar, and in the other 7.5 gm., although the amount of sugar which had been administered was less than 1/3 the amount a normal dog should be able to absorb in 3

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[3] Page, I. H., *J. Lab. and Clin. Med.*, 1923, ix, 194.

[4] Weiss, S., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 363.

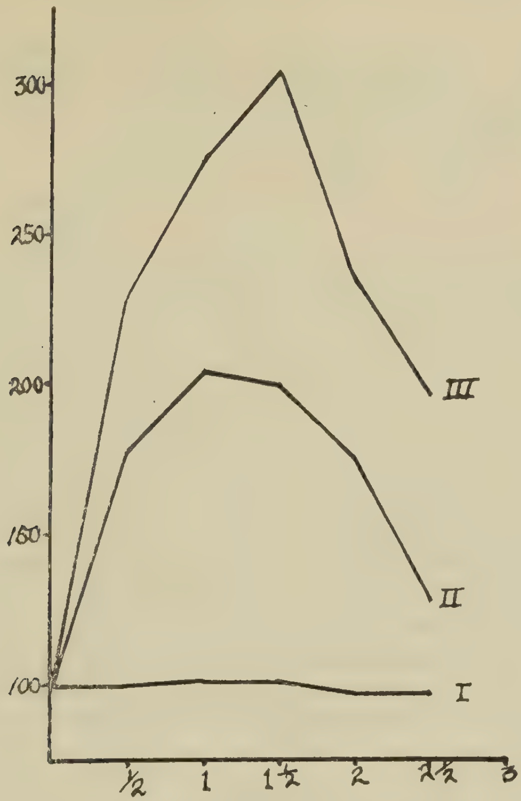

FIG. 1.

Ordinates = blood sugar. Abscissae = time in hours. Curve I = dog under amytal, sugar in stomach. Curve II = dog under amytal, sugar in duodenum. Curve III = dog unanesthetized, sugar in duodenum.

hours.[5, 6] A small fraction of this reducing substance was doubtless not sugar, but the reducing substance always found in the intestine; however, it can be safely said that 1/5 of the sugar remained unabsorbed in spite of the fact that the blood sugar had fallen to normal half an hour previously.

These experiments show that under amytal the pyloric sphincter is maintained so tightly closed that sugar introduced into the stomach can not pass into the duodenum, and if the sugar is placed directly into the duodenum absorption is diminished to a marked degree. This latter effect may, however, be merely a matter of diminished intestinal motility.[8] The closed sphincter and decreased intestinal

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[5] Fisher, G., and Wishart, M. B., *J. Biol. Chem.*, 1912, xiii, 49.

[6] Woodyatt, R. T., Sansum, W. D., and Wilder, R. M., *J. Am. Med. Assn.*, 1915, lxv, 2067.

[7] Cori, C., *J. Biol. Chem.*, 1925, lxvi, 691.

[8] Pottinger, Symptoms of Visceral Disease, 1922.

motility suggest paralysis of the parasympathetic autonomic nervous system, and is in line with the observation that vagus stimulation in dogs under amytal fails to lower blood pressure.[9]

4627

Skin Infection in Salmon Fishermen in Alaska.

H. N. MARCH AND MAX S. MARSHALL.

From the Department of Bacteriology, Medical School, University of California.

The occurrence of various types of skin infection among commercial fishermen has frequently been noted. Minute abrasions from the teeth or the spines of fish open the way frequently to infection. Fellers[1] has isolated a pyogenic streptococcus associated with fish and Klauder and his associates[2, 3] have noted on the east coast a specific skin infection due to the bacillus of swine erysipelas.

While giving medical assistance to the commercial fishermen at Bristol Bay, a part of the Bering Sea, one of us (H.N.M.) noted a type of skin infection which was typical and had uniformly constant symptoms. Although the fishermen do not distinguish between this and other infections, the disease has apparently been known to them for some time.

The disease appears to have a short incubation period. Pain, swelling, and a dark red discoloration develop simultaneously. If, at this stage, the area is examined closely, a tiny break in the skin may be noted from which a drop or two of clear or slightly cloudy serum can be expressed. There is general malaise, often a slight headache, and a temperature between 99 and 100. Swelling, or pain, or both may become quite marked, so that a lesion starting on a finger produces edema of the whole hand and sometimes of the wrist. Epitrochlear and axillary lymph nodes are commonly enlarged or tender. Lymphangitis is rarely visible. The infection spreads locally between the epidermis and the dermis, separating the two layers to form a lesion resembling a collapsed blister, containing a few drops of turbid serum. Improvement usually begins in 2 or 3 days, but the blister may spread for 5 or 6 days, with more or less burning and pain, the pain frequently extending up the arm. The period of disability is usually 7 to 10 days. The epidermis separates from the underlying tissue (it was usually cut away), the healed wound resembling an old water blister from a second degree burn, from the size of a dime to the size of a dollar. True pus is formed only with secondary infection.

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[9] Lieb, C., and Mulinos, M., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 709.

[1] Fellers, C. R., *J. Bact.*, 1926, xii, 181.

[2] Klauder, J. V., *J. Am. Med. Assn.*, 1926, lxxxvi, 536.

[3] Klauder, J. V., Righter, L. L., and Harkins, M. J., *Arch. Derm. and Syph.*, 1926, xiv, 662.

Smears were made on pieces of window glass when first reported, and on subsequent visits. A medium, prepared from frozen beef, and another prepared from raspberry gelatine, were prepared with the aid of faded litmus paper, and sterilized in a cannery retort. Cultures from lesions and from fish slime in these were brought to San Francisco for study.

Microscopic examination of the smears revealed in every case the presence of numbers of rather large Gram positive cocci, varying slightly but definitely in size and in their response to the stain, and occurring frequently in pairs, occasionally as short chains of 3 or 4 cells. They did not resemble streptococci, and did not seem typical for staphylococci. Every case showed these organisms in numbers, whereas only twice were other organisms noted, and these were not similar, nor were they present in any numbers.

Examination of the cultures revealed one tube in which the only viable organism was a coccus resembling exactly on morphological grounds the coccus observed in the directly prepared smears, including the tendency to arrangement in pairs, the slightly irregular staining, and the slight variation in size. No growth could be obtained from other cultures, with the exception of one made from the fish slime, from which several organisms were isolated, not certainly including the coccus above described.

This coccus grows luxuriantly on ordinary medium, presenting a slightly pigmented (a brownish yellow) opaque growth. Colonies are smooth, convex, round, several millimeters in diameter (24 hours). The optimum temperature appears to be 37°C. Broth cultures show an even turbidity in young cultures. Gelatin is very slightly liquefied, indol and H_2S are not produced, and neutral red and nitrate reduction tests were negative. Milk cultures show acid coagulation, and acid but no gas is produced in media containing dextrose, lactose, saccharose, maltose, or mannite.

Inoculation intraperitoneally into a guinea pig produced no reaction. Intradermal inoculations into a rabbit showed on the ear a progressive inflammation and some induration for several days, followed by absorption and healing. On the flank there appeared, following an inflammatory reaction, a hard swollen lump perhaps 5 mm. in height, and somewhat over a centimeter in diameter. A similar, but deeper, localized process resulted from a subcutaneous injection into a rabbit. The lesions appeared to reach a peak in 5 days, at which time incisions were made. These revealed encapsulated deep pockets of a caseous pus, from which pure cultures of the original organism could be secured. Thus far the disease has obviously not been reproduced, and the animal lesions might be explained by supposing the response to be due to an organism not necessarily connected with the human skin infection. However, the study reveals consistently the presence of the coccus described, and the presence of no confusing types. An attempt to reproduce the infection more accurately will be the subject for further investigation.

4628

On the Flavianates of Some Nucleinic Substances.

L. R. CERECEDO. (Introduced by Carl L. A. Schmidt.)

From the Division of Biochemistry, University of California Medical School, Berkeley

Due to the lack of dependable methods for their identification, the isolation of nucleinic compounds from biological fluids is not an easy matter. Among the precipitants which might be useful in the isolation of these substances, flavianic acid (1-naphthol-2-4-dinitro-7-sulfonic acid) seemed to be promising. Kossel and Gross[1] found that flavianic acid forms crystalline compounds with organic bases. Among other substances these workers prepared guanine flavianate.

We have prepared the flavianates of cystosine, 5-methylcystosine, 2-amino-4-methyluracil, guanosine, adenosine and cytidine.

It has been found (with O. H. Emerson) that cytidine flavianate is a very useful substance in the isolation of cytidine from yeast nucleic acid. Flavianic acid is more satisfactory than picric acid for decomposing cytidine nitrate. Although cytidine flavianate does not seem to crystallize from an impure solution, it forms beautiful crystals when fairly pure and is a very convenient substance for recrystallizing as it has a very high temperature gradient of solubility. The flavianate is easily decomposed by dissolving it in 5% sulfuric acid at 60° and extracting the flavianic acid with butyl alcohol from the warm solution. The cytidine sulfate obtained from the flavianate was extremely pure, and one recrystallization proved sufficient to obtain the base in beautiful crystals.

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[1] Kossel, A., and Gross, R. E., *Z. physiol. Chem.*, 1924, cxxxv, 167.

4629

Studies on the Physiology of Pyrimidines. The Intermediary Metabolism of Uracil.

L. R. CERECEDO. (Introduced by Carl L. A. Schmidt.)

From the Division of Biochemistry, University of California Medical School, Berkeley.

In an earlier paper[1] experiments were reported which showed that uracil and thymine, when fed in small amounts to dogs, are metabolized to urea. It was also shown that thymine glycol which is obtained on oxidation of thymine *in vitro*[2] is probably an intermediate step in the oxidation of this pyrimidine in the animal body. These results led the writer to investigate the fate of isobarbituric and isodialuric acids which are oxidation products of uracil *in vitro*[3] as possible intermediate metabolic products of uracil.

Steudel[4] reported feeding experiments with these two substances. He found that they were apparently completely oxidized.

Isobarbituric acid and isodialuric acid were fed to dogs maintained on a nitrogen equilibrium. In every case when isodialuric acid was administered, there was an increase in the urea output, suggesting that this substance was metabolized. In the case of isobarbituric acid, the increase in the urea output after feeding the substance was not as pronounced as after administering isodialuric acid. This may have been due to a lesser absorption of isobarbituric acid on account of its being less soluble.

These experiments seem to indicate that in the metabolism of uracil we are dealing with an oxidation resembling that *in vitro* as follows: Uracil → Isobarbituric acid → Isodialuric acid → urea + 3 C-atom compound.

In the course of these experiments we have observed a peculiar effect of these substances on the sulfur metabolism of the dogs. We found a distinct decrease in the output of inorganic sulfur on the day after feeding these pyrimidines. In some cases this decrease amounted to one-half of the amount ordinarily excreted. The writer is investigating this point further.

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[1] Cerecedo, L. R., *J. Biol. Chem.*, 1927, lxxv, 661.

[2] Baudisch, O., and Davidson, D., *J. Biol. Chem.*, 1925, lxiv, 233.

[3] Behrend, R., and Roosen, O., *Ann. Chem.*, 1889, ccli, 235.

[4] Steudel, H., *Z. Physiol. Chem.*, 1901, xxxii, 285.

4630

Effect of Protein Heat-Denaturization on the Precipitin Graph.

HAROLD C. SOX AND W. H. MANWARING.

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If 1 cc. horse serum is added to 19 cc. normal dog serum and the mixture is heated to 60° C. for several days, parallel titrations of the resulting products by means of ice-chest ripened anti-horse rabbit precipitin show a gradual flattening of the precipitin graph,[1] without appreciable change in the end reaction to "titer". The flattening of the graph is, therefore, taken as an index of specific protein denaturization. The stability of the end point suggests the relative reliability of the end reaction as a quantitative test.

The above tests are preliminary to an attempted interpretation of the altered precipitin graphs obtained with parenterally injected alien proteins.

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Parenteral Retention of Undenatured Foreign Proteins.

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Ice-chest ripened anti-horse rabbit precipitin contains: (a) a high-titer, highly active specific precipitin for horse proteins, and (b) a low-titer, relatively inert "non-specific precipitin" for canine proteins. Acting together these two precipitins presumably function as (c) a precipitin for hypothetical proteins of intermediary, "hybrid," or horse-canine specificity.

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[1] For technic and typical graphs see PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 14.

By the use of such antisera we have obtained precipitin graphs which suggest a 200% to 400% increase in hypothetical "hybrid" proteins in the canine circulation by the end of 18 days, with a 25% retention of these hypothetical intermediary products at the end of 3 months.[1] That the intermediary substances thus demonstrated are partially caninized horse proteins or their equivalent and not mere toxic increases in some normal non-specific canine factor is shown by our failure to obtain similar increases and retentions on intravenous injection of heterologous proteins (*e. g.*, beef serum).

Parallel with these tests we are following the parenteral history of the undenaturized residue of the injected horse proteins by means of antisera from which the canine and "hybrid" factors have been "absorbed". To prepare this altered precipitin 2 cc. crude antiserum is diluted with 7 cc. Ringer's solution, and 1 cc. normal canine serum then added. The mixture is incubated 2 hours, stored in the ice-chest over night, then passed through a Berkefeld filter. The "reduced precipitin" thus obtained gives no demonstrable reaction with normal canine proteins and presumably no reactions with proteins of dominant canine characteristics.

Our results to date indicate that undenatured horse proteins decrease rapidly in the canine circulation. But 12.5% to 25% of the routine initial dose is demonstrable at the end of 15 days, and but 1% to 3% by the 25th day. From 0.01% to 0.1% is present at the end of three months.

These figures agree with the orthodox conception of the parenteral history of chemically unaltered foreign proteins.

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A Flagella and Capsule Stain for Bacteria.

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From the University of California, Los Angeles, Calif.

The following method of flagella and capsule staining is offered as rapid, simple, and dependable. The method has been especially designed for staining *Bacillus proteus, Bacillus subtilis*, and the various members of the colon-typhoid group.

The procedure is as follows: 1. Make a thin smear of a 15-24 hour agar growth of the bacteria in a loopful of water on a clean slide. Air-dry. Do not heat. 2. Cover with mordant (5% tannic acid, 3 parts; 10% ferric chloride, 1 part) for 2 minutes. 3. Put 7 drops of mordant in a watch crystal or other small receptacle and add one drop of Ziehl-Neelson carbol fuchsin stain. Mix. Add one drop of concentrated hydrochloric acid. Mix. Add one drop of formaldehyde. Mix. 4. Pour off mordant from slide and cover smear with the mixture prepared in paragraph 3. Apply 7 minutes. 5. Wash in running water. 6. Cover with Ziehl-Neelson carbol fuchsin stain (Basic fuchsin, 10 gm.; 95% ethyl alcohol, 100 cc.; phenol, 5%; water, 1000 cc.) and steam gently for one-half minute. 7. Wash in running water.

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[1] For technic and typical graphs see PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 14.

The following observations are in order. A. Remove a small portion of the youngest marginal growth from an agar culture. The medium must not be dry. Add to droplet of water upon a slide. Allow this to stand a few minutes. Then transfer a loopful to a second slide. Work with this second slide. B. The mordant will keep indefinitely and thus can be prepared in quantity. The mixture prepared in No. 3 should be used fresh. C. The action of the mordant usually requires 2 minutes. D. Steaming of the stain must not be too severe. Instead of using the recommended stain in No. 6, the more powerful mixture of anilin gentian violet and carbol fuchsin may be preferred by some. E. Filtered mordant and stain yield better preparations than unfiltered materials.

The flagella stain described is a capsule stain as well. It shows the capsules of such organisms as *Diplococcus pneumoniae, Streptococcus fecalis,* and Friedlander's bacillus when these are grown in liquid protein rich media. It is a valuable routine capsule stain for the peritoneal exudate of white mice that have been killed by Pneumococci. The following procedure is recommended: 1. Add a loopful of the exudate to a loopful of water and spread thinly upon a slide. 2. Apply mordant as described in No. 2 for 10 seconds. 3. Wash in running water. 4. Apply cold diluted carbol fuchsin stain for 10 seconds. 5. Wash with water, blot, and examine.

The undiluted exudate, stained likewise, shows the capsules well.

4633

Effect of Milk, Lactose and Lactic Acid on Intestinal Bacteria and Protozoa.

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Diet has long been recognized as a factor in producing change in the intestinal flora, and the conclusions of Metchnikoff, Rettger and Cheplin,[1] Kopeloff,[2] and others, on the significance of aciduric bacteria within the intestine, have attracted considerable attention. Only a small amount of work, however, has been reported with reference to changing the intestinal protozoa by diet, Hegner[3] and Ratcliffe[4] showing a reduction of flagellates by feeding a high protein diet, and Kessel,[5] and Kessel and Huang[6] reporting a reduction in the numbers of amoeba when an exclusive milk diet was given. Beach[7] also advocates the use of lactose or dry skim milk for the treatment of coccidiosis in chickens.

Since lactose in sufficiently large quantities may produce a similar change in flora within the intestine to that produced by a milk diet, it is reasonable to assume that the lactose of the milk is the more important factor in bringing about the change. Since the end product of lactose in the intestine is lactic acid, it further appears logical to assume that the lactic acid alone would produce identical results. With this idea in mind, it was decided to compare the effects of milk, lactose and lactic acid in producing change in pH and in intestinal bacteria and protozoa.

For the initial observations, the experimental animals were placed on a general neutral diet for a period of 2 weeks, near the close of which time the feces and colon contents, freshly obtained, were subjected to the following determinations:

1. Examination for intestinal protozoa by smear method, both

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[1] Rettger, L. F., and Cheplin, H. A., The intestinal flora with special reference to the implantation of *B. acidophilus.* (New Haven, Yale Univ. press), 1921, 135.

[2] Kopeloff, N., Lactobacillus acidophilus. (Baltimore, Williams and Wilkins Company), 1926, xi, 211.

[3] Hegner, R. W., *Am. J. Hyg.*, 1923, iii, 180.

[4] Ratcliffe, Herbert L., *Am. J. Hyg.*, 1928, viii, 910.

[5] Kessel, J. F., *Univ. of Calif. Publ. Zool.*, 1924, xx, 489.

[6] Kessel, J. F., and Huang K'E-Kang, PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 388.

[7] Beach, J. R., *Hilgardia*, 1925, i, 167.

preliminary stains in iodine-eosin, and permanent stains in iron haemotoxylin being used. In addition, the feces were cultured in Ringers-egg-serum medium.

2. Determination of pH by the colorimetric method, using standardized dilutions.
3. Routine gram stains were made of the fecal bacteria.
4. Culture for the *B. coli*-aerogenes group, using Eosin Methylene Blue plates.
5. Culture for anaerobes using Robertson's meat medium.
6. Culture for acidurics was made on Bacto-Gelactose Whey agar plates. Differentiation between *B. acidophilus,* X-type, and other aciduric bacteria was made on the basis of the fuzzy type colonies.

Three chemical and bacteriological determinations were made before feeding experiments were begun. The feeding experiments were continued for a period of from 2 to 3 weeks, the same chemical and bacteriological determinations being repeated near the close of the experiment. Protozoological examinations of the rats were made at autopsy and the monkeys were given 6 or more follow-up examinations after the conclusion of the feeding.

In the study on hospital patients it was not possible to carry on the bacteriological culture work, and the determinations were limited to the pH, the gram stains, and the protozoological examinations.

The results of investigators in the past in administering lactic acid have not met with uniformity, though the generally accepted conclusion appears to be that lactic acid given alone in food or water has a negligible effect on the contents of the colon. To check this assumption, rats were fed only lactic acid soaked bread during a period of 2 weeks. Since, at the end of that time, there was no change either in the pH of the colon contents, nor in the type of bacteria present, it was concluded that lactic acid given free is not effective in producing the changes in flora.

Lacto-kelpol, a specially prepared emulsion of lactic acid in anhydrous agar and mineral oil, was extensively employed in treating colitis and also in combination with *B. acidophilus* therapy. The claim for this product was that the lactic acid was bound in the agar so that it passed through the stomach, thus reaching the intestine unchanged. It was thought desirable to use lacto-kelpol as a means of conveying the lactic acid to the colon, and in order to determine its effectiveness, feces of 7 monkeys and 15 human cases were tested for the presence of lactic acid, using a slight modification

TABLE I.

Showing the Results of Feeding Rats, Monkeys and Man With Milk, Lactose, and Lactic Acid (Lacto-Kelpol).

	pH		Gram+		Amoeba		Tricho-monas		*B. coli aerogenes*		*B. acidophilus* (X) fuzzy		Anaerobes	
	B.	A.	B.	A.	B.	A.	B.	A.	B.	A.	B.	A.	B.	A.
Rats			%	%										
Controls	6.5	6.8	10	10	5+	5+	5+	5+	556	740	6940	6020	F++P+	F++P+
Milk	6.3	6.5	20	95	5+	1+	5++	5+	2360	615	8475	19800	F+ P+	F++P+
Lactose—.1 gm. = 4 oz. daily for man	6.5	6.6	25	45	2+	1+	5+	5+	1850	6080	10980	10020	F++P++	F++P+
Lactose—2 gm. = 2½ lbs. daily for man	6.9	6.2	10	90	3+	3+	5+	1+	936	1076	3220	47700	F+ P++	F++P+
Lactic Acid (Lacto-Kelpol 1%)	6.7	6.5	10	60	5+	2+	5+	3+	1635	870	15190	36190	F+ P+	F+ P+
Milk and Lactic Acid (Lacto-Kelpol 1%)	7.2	7.0	10	80	0	0	5+	3+	7320	1430	2580	12920	F+ P++	F++P+
Monkeys														
Controls	6.8	6.8	10	10	2+	2+	2—	2—	1000	1500	2400	2800		
Milk only	6.7	6.5	10	85	3+	2+	—	—	3766	355	9000	24133	—	
Lactose—40 gm. = 1¼ lbs. daily for man	6.8	6.6	10	70	3+	3+	3+	3+	1270	1570	2144	3150	F+ P++	F++P+
Lactic Acid (Lacto-Kelpol 1%)	6.9	6.6	10	80	3+	3+	3+	3+	2272	4234	1540	6410	F+ P+	F+ P+
Milk and Lactic Acid (Lacto-Kelpol 1%)	7.8	6.7	10	80	2+	2+	2+	2+	3250	2190	9000	72000	F+	F++
Human														
Lactic Acid (Lacto-Kelpol 1%)	7.2	6.5	G—	G+	6+	4+	7+	5+	—	—	—	—	—	
Milk only					3+	2+								

B = Before feeding experiment. A = After feeding experiment.

of Uffelman's technique employed in testing gastric contents. No acid was demonstrable in these tests. Ten of the human cases and 4 of the monkeys were then given lacto-kelpol in addition to their regular diet. A test made in 7 days demonstrated acid in the feces while the controls were negative. It was, therefore, concluded that lacto-kelpol is a satisfactory means of conveying lactic acid to the colon.

From the accompanying table, a summary of experiments conducted together with their several controls, the following observations may be made.

(1) *Changes in pH.* Cannon and McNease[8] recorded a remarkable lowering of pH in their rats which were fed on an exclusive milk diet. Rettger and Cheplin,[1] however, were unable to demonstrate such a marked change and showed that the flora was altered even though great variations in pH were not apparent. In this investigation a lowering of pH is particularly noticeable in the human series given lactic acid (lacto-kelpol) only, where there is an average reduction in pH from 7.2 to 6.5, and in the monkeys which were given a combined diet of milk and lacto-kelpol, where the drop was from 7.8 to 6.7. One would conclude that in certain instances only an increase in aciduric bacteria is associated with a corresponding decrease in pH.

(2) *Gram Positive and Gram Negative Organisms.* The gram stain gives a rough index as to the increase or decrease of aciduric bacteria, but does not give specific information as to the type of aciduric organism present. In some instances *B. acidophilus,* X-type, has not increased appreciably when gram positive organisms have, though in such instances other types of acidurics or anaerobes were present.

(3) *Protozoa.* In considering the flagellates and the intestinal amoeba, it would appear that we are dealing with two very different types of organisms, and that, in all probability, Trichomonas grows with greater facility in an acid medium than amoebae. Knowles[9] feels that *E. histolytica* grows best in a slightly acid medium, but the results of Boeck and Drbohlav[10] do not warrant this conclusion *In vitro* cultures made in this study indicate that Trichomonas will grow well in a much wider range of pH than amoebae will.

Ratcliffe[4] says that a decrease in aciduric bacteria apparently does not affect trichomonads. From my preliminary findings with protozoa, this is exactly what one would expect, and I had hoped that the converse might be true and that an increase in aciduric bacteria might reduce the numbers of Trichomonas. This seems to be true within certain limits only, for though several of the human cases showed a reduction in the number of trichomonads, only 2 showed a complete riddance. All cases in which Trichomonads were reduced, also showed a lowering of pH, but all cases showing a lowering of pH did not give a corresponding reduction in flagellates.

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[8] Cannon, P. R., and McNease, B. W., *J. Inf. Dis.*, 1923, xxxii, 175.

[9] Knowles, Robert, An introduction to Medical Protozoology. (Thacker, Spink and Company, Calcutta), 1928.

[10] Boeck, W. C., and Drbolav, J., *Am. J. Hyg.*, 1925, v, 371.

The greatest reduction in amoebae has been encountered in the experiments with rats where the common species is one forming 8 nucleate cysts and, therefore, more closely related to *E. coli* than to *E. histolytica*. *E. coli* is more difficult to cultivate *in vitro* than *E. histolytica*, and the medium recommended for its cultivation is alkaline. It would, therefore, appear that *E. coli* cannot withstand as great a variation in pH as *E. histolytica*. In the rats, 8 of the 15 fed on milk, lactose, or lacto-kelpol, were cleared of their amoebae, the greatest reduction being in rats that received milk and lacto-kelpol. Only one monkey was cleared of amoebae in the series though 3 of the 9, or one-third of the human group cases given milk or lacto-kelpol, were cleared of *E. histolytica*.

(4) *B. coli-aerogenes Group*. The decrease of this group together with the change in pH, and the increase in aciduric bacteria, has not been constant, and when a reduction has occurred, the ratio of decrease has seldom been in the same proportion as the ratio of increase among the aciduric bacteria. *B. coli* does not entirely disappear as the aciduric bacteria increase, and the change in ratio is therefore due rather to the great increase of aciduric bacteria than to the decrease of *B. coli*.

(5) *Bacillus acidophilus, X-type*. Increase of this organism has been the most constant of any change noted in this study, and the same was most marked in the animals given an exclusive milk diet and in those given lactic acid (lacto-kelpol). An increase in aciduric flora among lactose fed animals, was noted only in those which were fed large amounts. Rettger and Cheplin[1] showed that 2 gm. of lactose, daily, per rat, are necessary to produce a marked increase in acidurics. This amount would approximate 2½ lbs. of lactose daily for man if body weights were compared. Rats given 0.1 gm. each, which would compare with 4 oz. daily for man, and monkeys given 40 gm. each, which would amount to 1¼ lb. daily for man, showed no increase in *B. acidophilus*, X-type, although they did show an increase in other aciduric organisms. With the exception of the rats given 2 gm. of lactose daily, the most marked and constant increase in *B. acidophilus*, X-type, was in the

animals fed lactic acid (lacto-kelpol), and in those fed a combination of milk and lacto-kelpol. Since there was no experimental implantation of *B. acidophilus,* it would appear that the predominating aciduric flora resulted merely from the addition of the lactic acid to the diet.

(6) *Proteolytic and Fermentative Anaerobes.* One may summarize the change in this group by stating that there is a slight decrease in the proteolytic anaerobes and a corresponding increase in the fermentative group, when milk, lactose, or lacto-kelpol is given.

Summary: An exclusive milk diet, lactic acid in lacto-kelpol, or large amounts of lactose added to a regular neutral diet, will usually produce a lowering of pH of the colon contents, an increase in gram positive organisms including an increase in *B. acidophilus,* X-type, and at the same time, in some instances will show a reduction in the number of intestinal trichomonads and amoebae.

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Influence of the Rate of Secretion on the Urea Concentration of Saliva.

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Observations of Hench,[1] Schmitz[2] and others have shown that the concentration of urea in human saliva obtained by chewing paraffin averages about 80% of that of the blood. The published figures from which this average has been obtained show wide variations in different individuals. Those of Hench[1] vary from 50 to 130%, those of Schmitz[2] from 58 to 128%, and unpublished observations made in this laboratory in 1921 from 58 to 110%.

Specimens of saliva secreted at slow and at rapid rates were obtained from each of 16 individuals, and their urea concentrations (urea plus ammonia) compared with that of blood taken at the same time. The slow specimens were obtained without stimulation, the rapid ones by chewing paraffin. (Table I.)

All the subjects show a lower urea concentration in the rapidly secreted specimens, the average difference between slow and fast

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[1] Hench, P. S., and Aldrich, M., *J. Am. Med. Assn.*, 1923, lxxxi, 1997.

[2] Schmitz, H. W., *J. Lab. and Clin. Med.*, 1922, viii, 78.

TABLE I.
Observations on Whole Saliva. Mean Values.

	Blood	Saliva.					
Subject	Mg. urea per 100cc	Rate—cc per min.	Mg. urea per 100cc	% blood urea	Rate—cc per min.	Mg. urea per 100cc	% blood urea
1	19.4	0.57	20.7	109	1.68	16.7	86
2	31.5	0.26	43.0	134	3.66	25.7	81
3	19.3	0.32	25.5	133	4.30	12.3	64
4	30.4	0.29	50.0	158	3.28	21.4	71
5	41.8	0.33	57.6	139	4.40	33.4	63
6	39.0	1.30	33.1	73	4.00	29.8	66
7	28.9	0.31	39.8	138	3.20	22.0	76
8	20.7	0.68	21.7	107	7.20	18.4	89
9	26.9	0.27	27.1	101	2.77	24.7	92
10	31.3	0.39	45.0	144	6.55	23.7	76
11	64.9	0.24	74.1	114	4.24	45.3	70
12	25.5	0.24	33.8	134	1.64	20.1	78
13	28.7	0.32	39.9	138	3.27	24.6	85
14	19.8	0.12	26.1	132	1.98	14.4	73
15	22.9	0.53	27.3	119	6.95	19.3	84
16	19.9	1.00	14.4	72	4.40	11.6	58
16		0.47	44.9	122	3.97	22.7	76

specimens being 46 mg. per 100 cc. The high figures obtained when the secretion rate was low are no doubt partly due to accumulated ammonium salts in the mouth, as noted by Hench. Since we could not be sure that this source of error was completely eliminated by

TABLE II.
Observations on Parotid Saliva. Mean Values.

	Blood	Saliva					
Subject	Mg. urea per 100cc	Rate—cc per min.	Mg. urea per 100cc	% blood urea	Rate—cc per min.	Mg. urea per 100cc	% blood urea
1	11.8	0.27	11.9	101	0.69	9.1	77
2	32.2	0.19	34.9	109	0.96	19.9	62
3	44.0	0.14	45.4	102	1.02	32.3	73
4	27.8	0.10	31.2	112	1.08	19.9	72
5	39.3	0.23	38.2	97	1.23	26.6	68
6	38.0	0.55	33.4	88	2.78	23.6	62
7	49.3	0.27	39.3	80	1.67	30.8	63
8	33.3	0.58	29.3	88	2.09	23.8	71
9	33.4	0.37	31.0	93	1.15	25.0	75
10	35.6	0.50	32.5	91	3.00	27.6	77
11	39.0	0.18	40.7	105	1.32	28.2	72
12	22.0	0.29	23.3	95	0.83	18.0	82
13	23.6	0.29	32.8	101	0.52	27.0	83
14	20.5	0.53	18.7	91	2.50	17.5	85
15	21.8	0.22	20.8	95	0.75	16.4	75
16	26.1	0.19	26.7	101	1.18	21.4	82
17	24.2	0.23	19.7	81	0.87	16.3	68
18	20.9	0.52	17.8	86	1.37	14.2	69
18		0.31	29.3	95	1.39	22.1	73

preliminary washings of the mouth, a similar series of observations was made comparing the blood urea with that of saliva obtained from one parotid gland. Such saliva was found to contain only a negligible amount of ammonia. Solutions of tartaric acid (0.05% and 0.5%) were used as stimuli. The results are given in Table II.

The secretion rates in this table can not of course be compared with those in Table I, but all subjects show a diminution in urea concentration when the secretion rate is increased, averaging 7.2 mg. urea per 100 cc.

4635

Duck Disease Studies: II. Feeding of Single and Mixed Salts.

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From the Hooper Foundation for Medical Research of the University of California.

One phase of the duck disease study previously outlined[1] is an investigation to determine the probability of "alkali poisoning" as the cause of this malady. This report deals with preliminary data obtained in the forced feeding of salts and mixtures of salts, such as are known to occur naturally in some of the disease areas.

Male and female ducks of the pintail species, weighing from

TABLE I.

	Salt	Gm. Fed	Result
1.	Sodium Chloride	1.57	Non-toxic.
2.	,, ,,	4.72	Non-toxic.
3.	,, ,,	6.00	Death in 4 days.
4.	,, ,,	7.90	Death in 36-45 hours.
5.	,, ,,	15.80	Death in 2 hours 25 minutes.
6.	Magnesium Chloride	0.40	Non-toxic.
7.	,, ,,	1.00	Non-toxic.
8.	,, ,,	1.50	Paralysis starting in 40 min. and lasting 5-6 hours.
9.	,, ,,	3.00	Paralysis starting in 30 min. and lasting 5-6 hours.
10.	,, ,,	3.60	Vomiting in 10 minutes, paralysis in 20 minutes, lasting 5-6 hours.
11.	,, ,,	4.50	Paralysis in 5 minutes and death in 15 minutes.
12.	Calcium Chloride	1.38	Non-toxic.
13.	,, ,,	4.80	Great depression in 15 minutes and death in 35 minutes.
14.	Sodium Sulphate	2.60	Non-toxic.
15.	Sodium Nitrate	0.50	Non-toxic.
16.	Magnesium Nitrate	0.20	Non-toxic.

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[1] Shaw, P. A., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 6.

TABLE II.

	Relative %							
Ion Exp. No.	1	2	3	4	5	6	7	8
Sodium	36.0	35.7	32.7	34.0	34.9	33.3	34.1	18.0
Chloride	42.0	37.0	44.0	44.0	37.0	39.6	45.0	31.0
Sulphate	12.0	10.5	10.9	11.0	10.5	15.9	18.0	27.0
Nitrate	10.0	8.8	9.1	9.5	8.8	8.7		
Calcium			3.3			0.8	1.0	0.8
Magnesium				1.5		0.8	0.9	0.8
Bicarbonate					8.8	0.9	1.0	0.8
Potassium								21.6
Nitrite		8.0						
Mgm. per dose	730	830	805	778	830	838	728	887
No. of doses	3	1	3	3	3	3	3	4
Interval between doses, hrs.	1.7	—	1.7	1.7	1.7	2.5	2.0	1.5
Total dose in gm.	2.2	0.83	2.4	2.3	2.5	2.5	2.2	3.5
Toxic period, hours	0		0	3-9	5-8	5-8	0	2—
Hours to death		1				8		6-16

600-800 gm. were used for the experiments. The salts were fed in solution ordinarily, by means of a pipette, but in a few instances were fed dry in capsules. Some data on single salts are shown in Table I and on mixtures in Table II.

The results indicate that less than 5 gm. of sodium chloride is non-toxic; while 6 gm. or more is lethal, the time required to produce death decreasing as the dose is increased. Toxicity appears after administration of 1.5 gm. magnesium chloride, but an amount approximately 4.5 gm. is required for a lethal dose. Calcium chloride totaling 1.4 gm. was non-toxic, while 4.8 gm. was lethal in 35 minutes.

The symptoms of sodium chloride toxicity were nausea, general depression, and torticollis. Magnesium chloride produced a typical paralysis, rendering the bird entirely helpless for the period during which the effect lasted. With calcium chloride the ante mortem symptoms appeared to be general depression rather than paralysis. Details of the symptoms produced, together with the blood and pathological changes, will form a separate report.

Data of particular significance were obtained from the administration of certain mixtures, a marked increase in toxicity being noted in those solutions which contained nitrate together with either magnesium or bicarbonate. It should be noted in Experiment 3 that calcium with nitrate did not produce a similar toxicity, nor was toxicity developed in Experiment 7 in which magnesium and bicarbonate were used but no nitrate.

The greatest toxicity was found in Experiment 2, in which sodium nitrite was added to the solution used in Experiment 1. In this connection it may be significant that qualitative nitrite tests made on the solutions, after standing in the laboratory for 2 weeks, gave negative results in Experiment 1 and positive results in Experiments 3, 4 and 5, showing a reduction of nitrate to nitrite apparently through the action of calcium, magnesium or bicarbonate ion, depending on the respective cases.

Toxicity was also obtained by substituting potassium for a portion of the sodium as indicated by comparison of Experiments 7 and 8. It is true that a larger total was fed in the case of the potassium mixture but it should also be noted that toxicity appeared shortly after administration of the second dose. In the 8 experiments listed in Table II the minimum dose was 0.83 gm. and the maximum 3.5 gm., 6 of the experiments using practically a constant amount of 2.2 to 2.5 gm. In all 8 cases the relatively non-toxic salts sodium chloride and sodium sulphate compose 76.0 to 97.1% of the total.

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Promotion of Healing by Benzoyl Peroxide and Other Agents.

R. A. LYON* AND T. E. REYNOLDS. (Introduced by C. D. Leake.)

From the Department of Pharmacology of the University of California Medical School, San Francisco.

This is a study designed in part to test the suggestion of the late Prof. A. S. Loevenhart that certain organic peroxides (benzoyl peroxide in particular) should be superior to anything now employed for the healing of skin lesions. This is because of their antiseptic action, without protein precipitation, by the slow but continuous liberation of "active" oxygen in contact with living tissue, and because of a supposed beneficial effect of this liberated oxygen on the healing process. Failure of these agents to coagulate protein should be a weighty argument in their favor, since it may be claimed that antiseptics which have this action delay healing to just that extent. Some evidence in support of these views was furnished by Loevenhart.[1]

Experimental: About 25 square cc. on the inner surface of the leg below the knee was shaved, washed and dried. By means of a small pipette, 0.1 cc. concentrated sulphuric acid was applied to each of 8 spots, 2 cm. apart, within the area. Each drop of acid was allowed to remain on the skin for 10 seconds, when it was wiped off. The spots became yellow and pained, and within 15 minutes the area became erythematous. The erythema subsided within 2 hours. Directly after the burning, the spots were treated by applying a thin layer of ointment to each as follows: *(a)* a widely advertised proprietary ointment containing zinc oxide, phenol, salycylates, and oils; *(b)* 10% scarlet red sulphonate in petrolatum; *(c)* 5% tannic acid and 5% ethyl aminobenzoate in petrolatum; *(d)* 1% butesin picrate (p-amino-benzoyl-butanol picrate); *(e)* 5% benzoyl peroxide and 5% ethyl aminobenzoate in petrolatum (this turns dark brown on standing); *(f)* 10% ethacaine (p-amino-benzoyl-ethanol benzoate) in petrolatum; *(g)* 5% benzoyl peroxide in petrolatum, and *(h)* petrolatum alone. Fresh applications were made daily for 10 days. The results of one such experiment may be summarized in part as follows:

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* Squibb Research Fellow.

[1] Loevenhart, A. S., *Therap. Monatshefte*, 1905, xii, 426.

Ointment	Duration of pain after anointment minutes	Size of Scar 3rd day after burn		Size of Scar 7th day after burn Diam. mm.	Size of Scar 12th day after burn Diam. mm.
		necrotic center Diam. mm.	hyperemic total Diam. mm.		
Proprietary zinc oxide	10	3	5	4 (no scab)	3.5 (no scab)
10% scarlet red	15	3	5	4 (no scab)	4.0 (no scab)
5% tannic acid with ethyl aminobenzoate	5	4	5	5 (scab)	4.5 (thin scab)
1% p-amino-benzoyl-butanol picrate	5	3	5	4 (punctate redness)	3.5 (punctate redness)
5% benzoyl peroxide with ethyl aminobenzoate	5	3	5	4 (thin scab)	3.5 (thin scab)
10% p-amino-benzoyl-ethanol benzoate	5	4	5	5 (thin scab)	4.0 (thin scab)
5% benzoyl peroxide	5	3	5	3 (thin scab)	3.0 (no scab)
Petrolatum	15	4	5	5 (thin scab)	5.0 (thin scab)

In another group of experiments a series of uniform skin burns were made by applying for 7 seconds at an initial temperature of 100°C. the tip of a steel rod 4 mm. in diameter. The same ointments were used and in the same way as above. Five days after the branding and beginning of the treatment, the scars in one of these experiments appeared as shown in Fig. 1. It may be noted in comparison with the others that in the burn treated with benzoyl peroxide the blister had subsided and the area of the scar had been reduced.

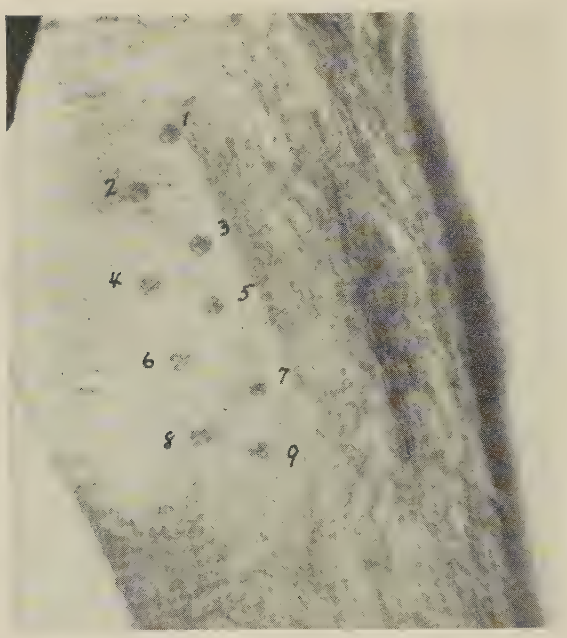

FIG. 1.

Skin lesions 5 days after applying for 7 seconds at an initial temperature of 100°C. the tip of a steel rod 4 mm. in diameter, and treating daily with (1) proprietary zinc oxide ointment, (2) 10% scarlet red sulphonate in petrolatum, (3) 5% tannic acid and ethyl aminobenzoate in petrolatum, (4) 1% p-amino-benzoyl-butanol picrate, (5) 5% benzoyl peroxide and ethyl aminobenzoate in petrolatum, (6) 10% p-amino-benzoyl-ethanol benzoate in petrolatum, (7) 5% benzoyl peroxide in petrolatum, (8) faulty burn, not uniform in size with rest, treated with 5% benzoyl peroxide in petrolatum, and (9) petrolatum.

The difficulty of judging from experiments such as these whether or not the healing process had been influenced by the various forms of treatment led us to begin a series of animal experiments in which we have attempted to use more satisfactory objective criteria, including the examination of histological specimens. These will be reported upon later.

While our experiments indicated the value of benzoyl peroxide ointments in promoting healing in certain types of skin injuries, clinical studies in connection with second degree burns failed to show their superiority to tannic acid dressings or to certain other ointments. However, it was found by Dr. H. Morrow and Dr. H. Miller in the Dermatology Clinic of the University of California Medical School, as well as by Dr. J. H. Woolsey in the Surgical Clinic, that a 10% benzoyl peroxide ointment in equal parts of petrolatum and lanolin is effective in clearing up chronic skin ulcers which have resisted treatment, in some cases for years.

Minnesota Branch.

University of Minnesota Medical School, October 30, 1929.

4637

Adsorption of the Ovarian Hormone from Urine.

H. O. WILES. (Introduced by J. F. McClendon.)

From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis.

In the purification of the ovarian (follicular) hormone, losses are experienced in filtration or dialyzation. Laquer, Hart and de Jongh[1] report adsorption on charcoal, talc and filter paper, but give no quantitative results.

1200 cc. of mixed urine from women in the last 2 weeks of pregnancy was centrifuged and divided into 4 equal portions. To one was added 30 cc. of a 10% acid calcium phosphate solution and to this was added 30 cc. of *N* NaOH. To another, double this quantity of phosphate was added. The 3rd portion was treated with 2.4 g. cellulose pulp. The 4th portion was left untreated. The content of each flask was put under suction to remove dissolved air, the flask filled with CO_2, loosely stoppered, heated, tightly stoppered and cooled, to minimize oxidation. The 3 portions were allowed to stand 20 hours at 10°. The phosphate was removed from the mixture by centrifuging, and was then dissolved in 35 cc. *N* HCl and made up to 300 cc. with water. The cellulose mixture was filtered on a Büchner funnel with a gum-rubber diaphragm over the top. The pulp mat was placed in a paper thimble in a Soxhlet extractor and extracted for 5 hours with ethyl alcohol under reduced pressure. The alcohol was then distilled off, leaving a small amount of watery residue which was diluted to 300 cc. The 6 watery solutions—untreated urine, 2 phosphate solutions and the urine treated by the first, the cellulose extract and the urine recovered by filtration—were extracted with three 60 cc. portions of ethyl ether, free from peroxide. The ether was distilled off and the residue taken up in boiling alcohol, cooled and centrifuged, and diluted with 1% NaCl solution. The potency was determined by the method of Allen and Doisy.[2] The weight of the non-volatile residue (a thick yellow oil) was determined and the weight per mouse unit calculated.

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[1] Laqueur, E., Hart, P. C., and de Jongh, S. E., *Deutsche Med. Wochschr.*, 1926, lii, 1331.

The results were as follows:

	Mouse units per liter	mg. per mouse unit
Urine, untreated	200-400	0.05-0.20
$Ca_3(PO_4)_2$ adsorbate	100-200	0.13-0.40
Residual urine	100-200	0.06-0.26
$Ca_3(PO_4)_2$, double quantity	200-400	0.08-0.16
Cellulose adsorbate	100-200	0.56-2.83
Residual urine	100-400	0.05-2.52

The results indicate that the hormone may be adsorbed quantitatively by calcium phosphate.

4638

The Psycho-galvanic Reflex and Polarization-Capacity of the Skin.

J. F. MC CLENDON AND ALLAN HEMINGWAY.

From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis.

It has been shown by Gildemeister that the physiological change in the skin constituting the psycho-galvanic reflex, is a change in polarization-capacity localized in the sweat glands. Those experimenters who offer other mechanisms of the reflex have worked with inadequate apparatus. We made a study of polarization-capacity, first of metal electrodes and secondly of the surface of red blood corpuscles. In order to distinguish between resistance and polarization-capacity we measured the resistance by means of a new Wheatstone bridge and an alternating current of one and a half million cycles per second. We are able to show that the resistance of the body does not appreciably change during the psycho-galvanic reflex. We devised an apparatus whereby the impedance of the body to direct current and high frequency current could be determined simultaneously, using a condenser to shield the high frequency current from the direct current and a choke coil to shield the direct current from the high frequency current. In an average of 10 simultaneous determinations the impedance of the human body to direct current fell 13.7% and to high frequency current 0.93%. In other words, the impedance of the body to high frequency current remains practically constant during the psycho-galvanic reflex. By means of a special switch we were able to determine the high frequency impedance or true resistance of the body, and then without disconnecting the bridge, by using a current of 1000 cycles per second and taking the value of the true resistance as constant, to determine the polarization-capacity of the skin. Polarization capacity is a capacity in parallel with a resistance; for example, in one determination, the high frequency impedance was 900 ohms, and the polarization-capacity of the resting skin was 0.035 micro-farads and 10,000 ohms. On stimulating another part of the body with a painful stimulus, the polarization-capacity changed to 8,800 ohms and 0.0357 micro-farads. Erroneous results are arrived at when collodion is used to mark out the area of skin measured, and this explains the erroneous idea that change is due to stretching of the skin. We used 2 adjacent fingers immersed in liquid electrodes and vaseline was used to mark out the area.

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[2] Allen, E., and Doisy, E. A., *J. Am. Med. Assn.*, 1923, lxxxi, 819.

4639

Use of Visking Sausage Casing for Ultrafiltration.

FRANK C. ANDRUS. (Introduced by J. F. McClendon.)

From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis.

Visking sausage casing is impermeable to native proteins but permeable to proteose. Three-fourths inch casing will not stand a pressure exceeding the osmotic pressure of blood colloids but one-half inch casings may be obtained. Ox blood serum diluted with one volume of water and saturated with toluene, was filtered through three-fourths inch casing by tying one end and sealing it with collodium and coiling 10 feet in an oval aluminum tray covered with a metal top sealed with vaseline. Two feet of the casing protruded from a hole in the cover and was supported vertically. The casing was filled with the serum and the pressure adjusted by the height of the vertical portion containing serum. A number of cc. per hour were filtered by a pressure little in excess of the osmotic pressure of the proteins. The ultrafiltrate contained the same concentration of chlorides as the serum, 70% of the calcium and 60% of the total phosphorus. The last 2 figures might have been different if the serum had not contained some albumose.

4640

Basal Metabolic Rate and Surface Area of Children.

D. A. COLLINS. (Introduced by J. F. McClendon.)

From the Laboratory of Physiological Chemistry, University of Minnesota.

Owing to the fact that R. E. Scammon had determined the surface area of many of the children in the Institute of Child Welfare, the basal metabolic rates of these children were determined. The measurements made by the Tissot-Haldane method were rejected, partly because there was no graphic record and irregularities in breathing could not be eliminated. The following determinations were made with the Benedict-Collins apparatus. The metabolic rates were not determined on the same days that the surface areas were measured by means of plaster casts and rates were determined on some children whose surface area was not measured directly. Boyd's and Scammon's formula (surface area $= 994.5\ W^{0.718}$) was found by them to apply to these children and, therefore, it is certain that a correct estimation of their surface area was made on the same day or within a very few days of the metabolic determinations. Therefore, these determinations are of special value as standards. Rates were determined successively on the same child several times a week. During some of the determinations muscle movements were observed and if these are eliminated the averages are as follows:

	Age		Cal. per sq. m.	
	Years	Months	per hour	
Males	3	3.5	52.2	
	4	7.5	56.2	
	4	11.5	49.4	
	5	—–	60.6	(Slight movements of the hands)
Females	5	1	48	
	5	2	51	

4641

Fox Encephalitis and Canine Distemper.

R. G. GREEN AND E. T. DEWEY.

From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, and the U. S. Bureau of Biological Survey.

A virus disease of dogs has been clearly defined by Laidlaw and Dunkin[1] as "canine distemper". A virus disease of foxes has also been defined by Green and co-workers,[2, 3] which, previously described as fox distemper, has been called "epizootic fox encephalitis". Through the kindness of Laidlaw and Dunkin, the viruses identified in the 2 investigations have been exchanged for comparative study.

While various methods of comparison have shown the 2 viruses to be distinct, the susceptibility of the ferret to the one and not to the other proves conclusively that epizootic fox encephalitis and the canine distemper described by Laidlaw and Dunkin are distinct entities. For the experiments here reported, young foxes and ferrets raised in careful quarantine were available. The fox encephalitis virus was a brain and spinal cord emulsion prepared from the pooled brains of 20 foxes dying of the experimental infection. The dosage used for both foxes and ferrets was 1 cc. of a 5% emulsion. This dosage was, then, enormously greater, in proportion to body weight, for the ferrets than for the foxes. Five fox pups 5 months old and 5 ferrets 2½ months old were injected subcutaneously with the same preparation of virus. The foxes showed a typical experimental infection of encephalitis, one dying on the fourth day, one on the fifth day, and 2 on the sixth. One fox showed grave symptoms on the fourth and fifth days but recovered. The 5 ferrets, during the same period, showed no signs of infection. Their condition was carefully noted from the eighth to the fifteenth day after injection, this being the period during which ferrets die from the virus of Laidlaw and Dunkin. At no time were any symptoms of disease noted.

The virus obtained from Laidlaw and Dunkin was established in our laboratories in both ferrets and foxes. Our observations on the great susceptibility of the ferret to this virus agree with those of Laidlaw and Dunkin. For the injection of the ferrets previously inoculated with fox encephalitis virus, we used a 5% ferret spleen suspension. Thirty-six days following the injection of our encephalitis virus, 3 of the 5 ferrets were inoculated with the canine distemper virus of Laidlaw and Dunkin. Two of these ferrets died on the eleventh day and one died on the twelfth. The remaining 2 ferrets were given the inoculation of canine distemper virus on the forty-eighth day after the injection of the encephalitis virus, and both succumbed to the disease 11 days after the inoculation.

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[1] Laidlaw, P. P., and Dunkin, G. W., *J. Comp. Path. and Therap.*, 1928, xli, 209.

[2] Green, R. G., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 677.

[3] Green, R. G., Ziegler, N. R., Dewey, E. T., and Shillinger, J. E., *Comp. rend. des seances de la Soc. de biol.*, 1929, c, 327.

It is concluded that epizootic fox encephalitis and canine distemper may be distinguished by a test of the susceptibility of ferrets. This animal is not susceptible to fox encephalitis but, as a rule, succumbs when inoculated with the canine distemper virus of Laidlaw and Dunkin. The injection of fox encephalitis virus into ferrets gives no immunity against subsequent injection of the canine distemper virus.

4642

The Irritability and Chronaxie of the Small Bowel.

H. H. DUKES. (Introduced by Walter C. Alvarez.)

From the Department of Veterinary Investigations, Iowa State College, Ames, Iowa, and the Division of Medicine, The Mayo Clinic, Rochester, Minnesota.

In view of Lapicque's claim that the chronaxie is one of the best measures of the excitability of a muscle we were curious to see what this "threshold of duration" is for the mammalian small intestine, and if it is graded from duodenum to ileum. Unfortunately, in the time that I could give to the problem no conclusion could be reached except that with both galvanic and faradic tetanic stimuli the time required in order to get a response varied between 0.1 and 0.6 second. In 3 animals the chronaxie appeared to be graded downward with short intervals in the duodenum and long ones in the ileum, and in one there was no gradation. The problem will have to be studied further probably with a different type of apparatus. I used the electrodes and technic described by Alvarez and Hosoi.[1] The duration of current flow was determined by 2 keys which were opened either by a Lucas pendulum[2] or by a gravity operated arm, the descent of which was regulated by a dash-pot. The Lucas pendulum with the keys separated as widely as possible delimited an interval which usually was too short for effective stimulation.

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[1] Alvarez, W. C., and Hosoi, Kiyoshi, *Am. J. Physiol.*, 1929, lxxxix, 182.

[2] Lucas, Keith, *J. Physiol.*, 1907, xxxv, 310.

With such a long chronaxie as was found it is curious that the neuromuscular wall of the bowel should respond at all to the rapidly interrupted faradic current.

During the course of these experiments it was necessary to measure the threshold for faradic stimulation and the opportunity arose of checking the recently published observations of Alvarez and Hosoi. Fig. 1 shows that a gradient of irritability similar to the one described by them was found in 12 out of 17 rabbits. In 2 the irritability was low and about the same in all parts of the bowel, and in 3 the gradation was irregular. In spite of the fact that I used their apparatus and their technic the thresholds obtained by me tended to be higher than those obtained by them and several were higher than any they obtained. On the whole their results are confirmed and the possibility remains that the animals with a flattened or irregular gradient of irritability were diseased and abnormal.

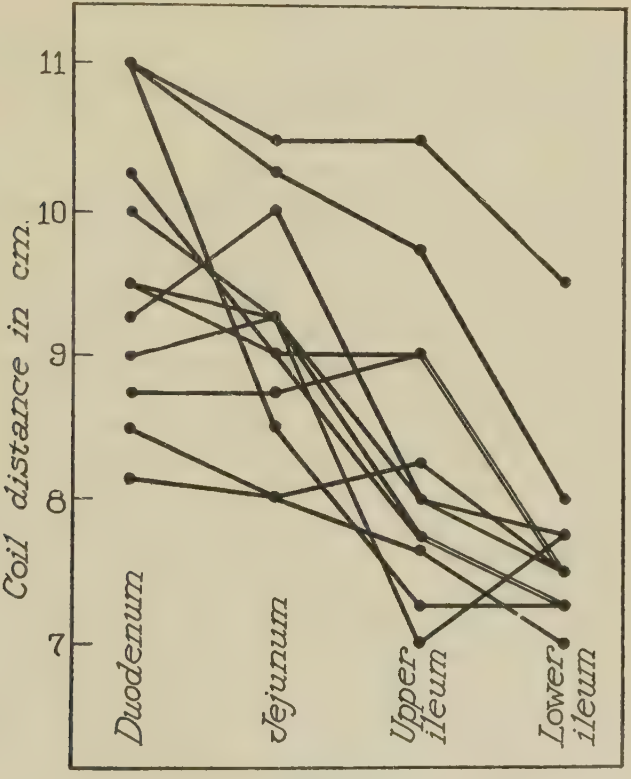

FIG. 1.

Gradient in irritability in the bowel of the rabbit. Ordinates represent coil distance in centimeters and abscissas represent four different regions in the bowel. Lines connect readings on one animal.

New York Meeting.

New York Academy of Medicine, November 20, 1929.

4643

The Form of Dye Penetrating the Cell as Determined by the Glass Electrode.

MARIAN IRWIN.

From the Laboratories of The Rockefeller Institute for Medical Research, New York.

Measurements with the glass electrode show that cresyl blue in the form of salt* decreases the pH value of distilled water and in the form of free base* increases it. The free base also increases the pH value of the sap freshly extracted from the vacuoles of living cells. This difference in the behavior of the dye salt and the free base enables us to determine whether free base of cresyl blue penetrates into the vacuoles of living cells of *Nitella*.

The pH value of the sap of *Nitella* (obtained from Maryland) was measured by means of the glass electrode immediately after extraction at about 25°C. The duration of each measurement was about 5 minutes. The pH value of the sap of the control cells was pH 5.77. Using indicator methods previously employed[1] with brom cresol purple (appearing yellowish when dissolved in distilled water) the sap from the control cells was only 0.1 pH lower. Each measurement was made immediately after extraction and took about 5 minutes. As the dye penetrates, the pH value of the sap is gradually increased to the extent of 0.5 pH or more.

The same concentration of brilliant cresyl blue produces approximately the same increase in pH value whether it penetrates the vacuole of a normal cell or is added in the form of free base *in vitro* to freshly extracted sap.

The work is being continued.

The measurements in distilled water were made by Dr. A. E.

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* Cresyl blue (prewar Grübler) is purchased in form of salt. The free base, free from impurities, may be obtained by shaking the aqueous solution with chloroform.

[1] Irwin, M., *J. Gen. Physiol.*, 1925-26, ix, 240.

Mirsky[2] while those in the sap were made by Dr. M. Dole.[3] I wish to thank them for their kindness and courtesy.

4644

The Isoelectric Point of the Dick Toxin.

A. P. KRUEGER AND H. T. TAMADA. (Introduced by J. H. Northrop.)

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

In the course of experiments having for their purpose purification of Dick toxin it became desirable to ascertain the electrical charge carried by the toxic material. This point seemed worthy of investigation not only because of our own immediate needs for such information, but also because the electrical charge borne by a toxin is an important factor in filtration, adsorption, flocculation, etc.

The method described by Krueger, Ritter and Smith,[1] based upon electrophoresis of the test substance into agar and subsequent resuspension in some appropriate menstruum, was employed. Toxin* of high titre was adjusted to the required pH value with N/10 NaOH or HCl, placed in the two cells of the apparatus and exposed to a current of 70-85 v. and 5-12 milliamps. for 20 hours. At the end of this time the agar cylinders from anode and cathode were removed and triturated with 10 cc. normal saline solution for 1 hour. The extracts so obtained were then made up in 1/100 dilutions with normal saline and injected in quantities of 0.1 cc. into the skin of individuals known to be Dick positive. Each test patient also received a control injection, *i. e.,* 0.1 cc. of a 1:10,000 dilution of toxin previously adjusted to the pH at which the test was run, maintained at this C_{H+} for the same length of time as the toxin in the apparatus and neutralized before injection. This injection served to detect inactivation of the toxin by acid or alkali and also furnished us with a criterion by which to read the other skin reactions. We found it necessary to employ in the cataphoresis cells a gel containing not more than 0.4% purified agar. Percentages higher than this did not permit the toxin to migrate.

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[2] Mirsky, A. E., and Anson, M. L., *J. Biol. Chem.,* 1929, lxxxi, 581.

[3] MacInnes, D. A., and Dole, M., *J. Gen. Physiol.,* 1928-29, xii, 805.

[1] Krueger, A. P., Ritter, R. C., and Smith, S. P., *J. Exp. Med.,* 1929, (in press).

* Dr. Gladys Dick very kindly supplied us with large quantities of toxin having a titre of 12,500 S.T.D.'s per cc.

TABLE I.
Electrophoresis of Dick Toxin in Broth.

pH	Hours current passed	E.M.F.	Milliamps.	Skin reaction 0.1 cc. intracutaneously			Nitrogen content. Mg./cc. of agar extract	
				Toxin control 1/10,000 dilution	Anode sample 1/100 dilution of extract	Cathode sample 1/100 dilution of extract	Anode sample	Cathode sample
5.0	21	75	5.5	(+)	(—)	(+)	0.066	0.174
6.0	20	80	5.0	(+)	(—)	(+)	0.068	0.134
6.5	20	75	9.0	(+)	(—)	(+)	0.096	0.128
7.0	20	82	8.0	(+)	(—)	(+)	0.087	0.172
7.0	20	85	12.0	(+)	(—)	(+)	0.107	0.138
7.5	21	80	6.7	(+)	(+)	(—)	0.076	0.202
7.5	20	76	8.0	(+)	(+)	(—)	0.081	0.210
8.0	20	75	9.0	(+)	(+)	(—)	0.173	0.098
8.0	21	80	7.6	(+)	(+)	(—)	0.165	0.110
9.0	20	80	7.0	(+)	(+)	(—)	0.144	0.081
10.0	20	85	8.0	(+)	(+)	(—)	0.173	0.106

The skin-test data presented in Table I indicate that the isoelectric point of Dick toxin lies between pH 7.0 and pH 7.5. Judging from the nitrogen content of the anode and cathode samples, however, the reversal in electrical charge occurs between pH 8.0 and pH 7.5. This anomaly is explained by the results of a series of identical experiments run with broth alone (Table II). Here it is apparent that the shift in nitrogen values takes place between pH 8.0 and pH 7.5 and it is probably the migration of non-toxic, nitrogen-containing compounds normally present in broth that causes the difference noted above. The control series in Table I is of interest in that it demonstrates the resistance of Dick toxin (in relatively high concentration) to changes in C_{H+} from pH 5.0 to pH 10.0.

TABLE II.
Electrophoresis of Broth without Toxin.

pH	Hours current passed	E.M.F.	Milliamps.	Nitrogen content. Mg./cc. of agar extract.	
				Anode sample	Cathode sample
5.25	24	70	5.5	0.042	0.091
6.0	24	70	5.2	0.061	0.130
7.0	24	65	7.0	0.042	0.160
7.5	25	66	4.8	0.040	0.138
8.0	24	65	6.0	0.118	0.056
9.0	26	65	10.5	0.161	0.038

Since we were not working with a pure toxin but with a toxin-broth mixture we have no reason to suppose that the determined isoelectric range is that of the toxin itself. There is the possibility that the toxin exists in physical or chemical combination with some broth constituent and that it is this aggregate which migrates in the electric field. Such a micella may well have quite a different isoelectric point from that of the pure toxin.

4645

Antemortem Basopenia as an Index of Resistance.

A. E. CASEY. (Introduced by L. Pearce.)

From the Laboratories of The Rockefeller Institute for Medical Research.

In a recent study of rabbit's blood[1] it was shown that the basophile level before inoculation seemed to bear some relation to the course of infection with *Treponema pallidum* and it was suggested that the basophile count might be used as an index of the resistance of the rabbit to this disease. During the same period (1927-28-29) blood counts were made upon 44 rabbits within 10 days before death, and it was found that all except 3 had both an absolute and relative basopenia. This agreed with the previous association of low blood basophiles with low resistance and high blood basophiles with high resistance. The rabbits were the fatal cases in the groups used for diverse experiments in which antemortem counts were made and represent approximately 20% of the total number of animals used. 32 of the 44 died from the effects of a transplantable neoplasm and the remaining 12 from other causes. 8 of the 44 animals were killed when in a dying condition; the rest were found dead.

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[1] Casey, A. E., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 670.

The differential counts were made with the neutral red supravital technic upon blood taken from a marginal ear vein. In all examina-

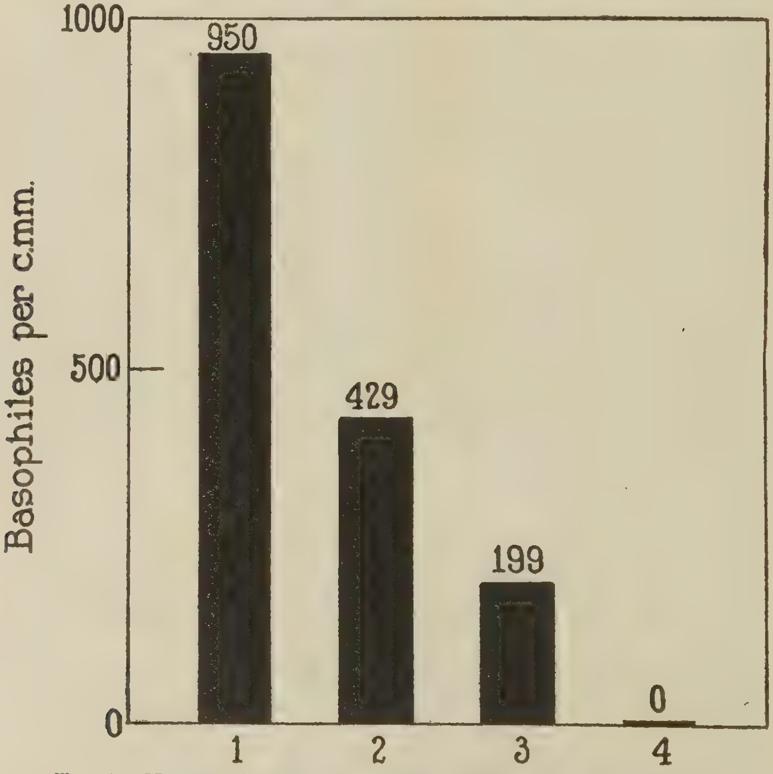

Fig. 1. Normal Contrasted with Antemortem Basophile Level.

1. Average level of basophiles in 174 normal rabbits during the same period.
2. Average level of basophiles in 32 tumor rabbits within 10 days before death.
3. Average antemortem level of basophiles in 12 rabbits dying of other causes.
4. Absence of basophiles 5 minutes after death in 2 tumor rabbits.

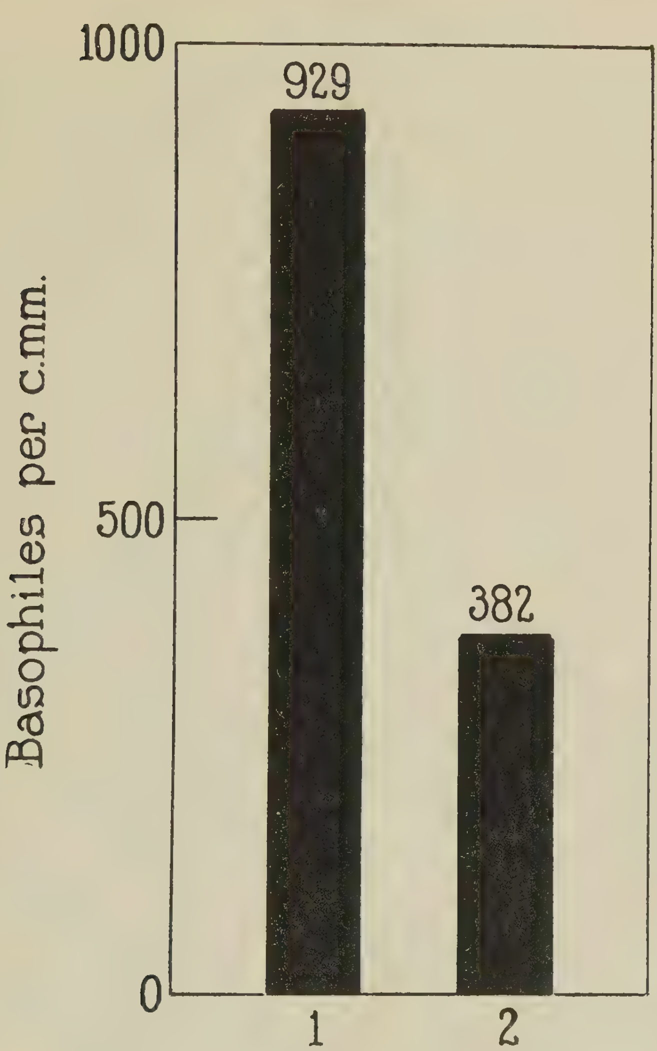

Fig. 2. Comparison of Preinoculation and Antemortem Basophile Levels.
1. Before inoculation. 2. Before death.

tions at least 100 cells were counted. In the instance of the postmortem counts made on 2 tumor animals 5 minutes after death, the blood was taken from the heart and 400 white cells counted in the first animal and 600 in the second. Total white counts were made at the same time as the differential. Most examinations were made at regular intervals and the time between the last count and the animals' death varied from one to 10 days with an average of 2.3 days.

The average basophile count of 950[2] per cmm. of blood obtained from 1110 counts upon 174 normal animals during the same period is used as a basis for comparing the results of the antemortem counts (Fig. 1). In the 44 animals within 10 days prior to death, the blood basophiles averaged 366 per cmm. The average antemortem count of the 32 animals which died from tumor was 429 per cmm. and in those dying from other causes was 199 cmm. of blood. In the case of the 2 rabbits in which the blood was examined 5 minutes after death, no basophiles were found in counting 1000 white cells. This, of course, can not be considered sufficient proof of the absence of basophiles in the blood of rabbits found dead from disease conditions but merely an indication of a trend.

From an examination of Figure 1 alone, it might be argued that the 44 animals under consideration had had a basopenia before inoculation and that it was not a condition incident to the period before death. The preinoculation counts of 19 of these rabbits, 18 of which were inoculated with tumor, showed an average level of 929 basophiles per cmm. as determined from an average of 10 counts for each animal (Fig. 2). The antemortem basophile level based upon the average of all counts made within 10 days of death (1 to 4 counts) was found to be 382 basophiles per cmm. (Fig. 2), demonstrating that there was actually a reduction in the blood basophiles before death. In this group of 19 rabbits only one had an antemortem basophile level of more than 600 basophiles per cmm.

It must not be interpreted, however, that basopenia necessarily indicates impending death because a low basophile count of comparable degree does occur under other circumstances. But from these observations and those previously reported, it would appear that the condition is associated with a state of low resistance. The exact relation of basopenia to the state of low resistance, whether of a causal or a merely concomitant nature, is not known.

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[2] Pearce, L., and Casey, A. E., Studies in the Blood Cytology of the Rabbit. I. Blood Counts in Normal Rabbits. In press.

4646

Survival of Vaccine Virus Separated from Living Host Cells by Collodion Membranes.

R. S. MUCKENFUSS AND T. M. RIVERS.

From the Hospital of The Rockefeller Institute for Medical Research.

The fact that vaccine virus is capable of multiplication in the presence of living host cells[1,2] cultivated *in vitro* has been known for a number of years. Reports concerning the growth of the virus on lifeless media have not been substantiated. Although it has, as yet, been impossible to cultivate the virus of vaccinia in the absence of living cells, no one definitely knows what function these cells serve in the cultures. Recently, Maitland and Maitland[3] demonstrated that vaccine virus increased in a medium consisting of minced kidney suspended in a mixture of serum and Tyrode's solution. The development of this fluid medium, in which living cells persist for at least 5 days,[4] has made possible experiments designed for an intensive study of the relation of vaccine virus to host cells.

Collodion sacs, prepared according to the method of Northrop and Kunitz,[5] were placed over the lower ends of small open tubes that fitted into larger test tubes. The small inner tubes with their attached sacs and the larger outer tubes were sterilized separately in an autoclave, after which the former were placed in the latter, thus forming 2 compartments separated at the lower level by the membrane alone.

Levaditi neurovaccine prepared in the testicles of rabbits was used. Emulsions of the infected testicles, from which cells were thoroughly removed, were diluted with rabbit serum and Tyrode's solution and then placed on one side of the collodion membrane, while a mixture of minced normal kidney, normal serum, and Tyrode's solution was placed on the other side. Vaccine virus under these conditions, although separated from living cells, survived a 4-day incubation at 37°C., while the virus in controls—a mixture of virus, serum, and Tyrode's solution incubated alone in test tubes—became inactive.

Kidney tissue, killed by freezing and thawing, mixed with serum

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[1] Harde, E. S., *Ann. Inst. Pasteur,* 1916, xxx, 299.

[2] Parker, F., Jr., and Nye, R. N., *Am. J. Path.,* 1925, i, 325.

[3] Maitland, H. B., and Maitland, M. C., *Lancet,* 1928, ii, 596.

[4] Rivers, T. M., Haagen, E., and Muckenfuss, R. S., *J. Exp. Med.,* 1929, l, 181.

[5] Northrop, J. H., and Kunitz, M., *J. Gen. Physiol.,* 1925-26, ix, 351.

and Tyrode's solution did not support the survival of vaccine virus at 37°C. for many days. If, however, a mixture of vaccine virus, kidney tissue killed by freezing and thawing, serum, and Tyrode's solution was placed on one side of a collodion sac, and a preparation of living minced kidney tissue, serum, and Tyrode's solution was put on the other side, the survival of the virus was far better than that in the first set of experiments described above. In fact, there seemed to be complete survival, if not multiplication of the virus. The results were so striking that further work is in progress to ascertain the nature of the factors responsible for the survival of the virus, and to determine if multiplication of the virus can take place under such conditions.

4647

Observations Suggesting a Local Factor in Pathogenesis and Healing of Rickets.

A. F. HESS, M. WEINSTOCK, H. RIVKIN AND J. GROSS.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

The pathogenesis of rickets is a moot question. By many it is believed that this disorder is entirely of systemic origin and that the local lesions at the epiphyses are secondary in nature. This point of view has been fortified by the demonstration that a low concentration of inorganic phosphorus in the blood is one of the most constant phenomena of rickets in infants and in animals. There can be no doubt of the participation of the systemic factor. However, as we have pointed out from time to time, rickets may come about occasionally in infants and in animals in spite of the fact that the calcium and the phosphorus in the blood have remained at normal levels. In rats this relationship was noted most often when about 10% dried milk was added to the standard rickets-producing ration. Furthermore, it was observed that the lesions of rickets may fail to become manifest in some infants and animals which are poorly nourished, although the phosphorus in the blood is definitely below the non-rachitic level. This incongruity was noted in some instances among rats which had been fed the Sherman-Pappenheimer diet, which is deficient in several nutritional factors.

Last year this subject was again brought to our attention by the occurrence of a mild degree of rickets in some infants, notwithstanding the fact that they were well nourished and that the calcium and phosphorus content of the blood remained practically unaltered. These infants should have been fully protected as they were fed irradiated dried milk. On further investigation, it was found, however, that this milk had been insufficiently activated, due to a technical error in the course of irradiation.

More recently we found that the subject was open to investigation by means of animal experiment. Our first step was to induce rickets by means of a diet in which there was a marked excess of calcium. This was accomplished by substituting yellow corn meal for the yellow corn in the standard Steenbock rickets-producing ration. By this means the ratio of Ca :P was rendered exceptionally high, about 11.5 :1, due to the low phosphorus content; the excess of base over acid was about 520 cc. of 0.1 N alkali. The next step was to administer an antirachitic agent. It was found that in spite of very large amounts of cod liver oil or of irradiated ergosterol, the rachitic lesions in the epiphyses remained unaffected, whereas the blood showed normal or even excessive concentrations of inorganic phosphorus and calcium; in some instances the ratio was normal, in others the one or the other element was disproportionately great.

In many instances in which rickets was brought about by this means, the multiple of Ca × P of the blood was above rather than below normal. It has been held that if this multiple is as high as 45, rickets will not develop. In some animals this multiple was even as high as 80 or 90 and nevertheless rickets persisted.

The presence of rickets was diagnosed by the typical radiographic picture. In many instances, the diagnosis was confirmed by means of histological examination. In still others it was further substantiated by an analysis of the bones. The ash was found to be low; instead of the normal percentage of about 50, it was approximately 30%.

Not only was a form of "local rickets" developed, but, as stated, these lesions were extraordinarily resistant to treatment with specific antirachitic measures. Healing could not be brought about by cod liver oil, even in amounts as high as 40 times the curative dose. Various standard brands of cod liver oil were fed. Nor was egg yolk effective in bringing about healing; 0.5 gm. was fed daily. Irradiated ergosterol also proved ineffective, even when 200 or 20,000 times the minimum curative dose was fed daily. It may be added that when very large amounts of irradiated ergosterol or cod liver oil were fed, the percentage of ash in the bones was lower than when lesser quantities were given. Ultraviolet irradiation was also ineffective in bringing about healing. For this purpose monochromatic rays were used; in one experiment a wave length of 2967 Ängstrom units of high intensity was employed. In order to assure ourselves that these antirachitic measures did not lack potency, we made control tests with the standard Sherman-Pappenheimer ration and found that on this diet healing could be readily induced. Moreover, if the calcium ratio in the diet was reduced to 9.5 instead of 11.5, moderate healing could be brought about. Furthermore, if $CaCl_2$ was substituted for $CaCO_3$ in equimolar amounts, it was found that the specific agents exercised a curative effect.

Although the rachitic lesions were but little affected by antirachitic agents when they were given for our usual test-period of 9 days, they underwent healing to some degree when these agents were continued for twice this length of time. In other words, the local factor which inhibited calcification was gradually overcome by the prolonged action of potent antirachitic measures.

These clinical observations and animal experiments combine to show that under certain conditions a local disturbance at the epiphyses may play the determining rôle in the pathogenesis and in the cure of rickets. Under such circumstances, the most potent antirachitic agents fail to exert their usual activity, notwithstanding the fact that the concentration and the ratio of the phosphorus and calcium in the blood are at normal levels.

4648

The Effect of Restricted Diet and Suprarenalectomy on Experimental Tuberculosis in the White Rat.

M. MAXIM STEINBACH. (Introduced by F. P. Gay.)

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

It has been previously demonstrated[1] that the albino rat enjoys complete immunity to infection by human tubercle bacilli in spite of the fact that the bacteria remain alive for indefinite periods and apparently multiply in the host.

In the first of these new experiments 36 rats were used and were divided into 3 groups. Twelve of these were inoculated with human, 12 with bovine and 12 with avian strains respectively of the tubercle bacillus. Six of each of these groups were on a bread and water diet and 6 on a full maintenance diet. Throughout all the experiments the dose used was 1 mgm. diluted with 1 cc. of saline and injected intraperitoneally. The strains were all pathogenic, H. 37, B. 1, and Avian, "pathogenic," and were obtained from Trudeau Sanitarium.

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[1] Ornstein, George G., and Steinbach, M. Maxim, *Am. Rev. of Tuber.*, 1925, xii, 1.

Of these animals those given the human and bovine strains, when autopsied after a suitable interval, showed no macroscopic or microscopic evidence of tissue reaction. On direct smears and tissue stains many of these animals showed the presence of the tubercle bacillus. Most of the animals, however, (82%) which had been inoculated with the avian strain, showed at autopsy extensive macroscopic tuberculosis. The spleen and liver seemed especially involved. These latter results agree with those of Cobbett.[2]

Each group inoculated was carefully controlled by rats fed on a normal diet. The control animals inoculated with the human strain, at autopsy were, as expected, negative. The bovine group was likewise found negative at autopsy. Of the control group inoculated with the avian strain, 50% were found at autopsy to have extensive tuberculous lesions.

We may then conclude from these experiments that the white rat is immune to the human and bovine strains of the tubercle bacillus even when its resistance is lowered by a deficient diet (white bread and water), but is susceptible to the avian strain. It was noted further that a greater percentage of infections took place among those fed on a restricted diet than among those normally fed.

On the basis of these conclusions, it was decided to investigate the possibility of infection with the bovine as well as the avian strain in animals whose resistance was lowered by bilateral suprarenalectomy. A large number of animals were suprarenalectomized, and were subsequently inoculated on the fifth day post-operative with either bovine or avian tubercle bacilli. Most of the animals died soon after. Of those that remained alive, 16 had been inoculated with the bovine organism and 5 with the avian.

Of the 16 animals in the bovine group, 12 survived for periods ranging from 7 to 22 days. Of these 12, all but one showed definite macroscopic or microscopic (or both) evidence of tubercle formation, and tubercle bacilli were demonstrated in the tissues. The remaining 4 in this group were sacrificed. Three of these showed definite macroscopic lesions. In the fourth, no lesions could be found.

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[2] Cobbett, Lewis, Causes of Tuberculosis, Cambridge University Press, 1917, 449-454.

We may say, therefore, that of the 16 animals all but 2 showed definite evidence of tubercle formation as the result of infection with the bovine strain of tubercle bacilli.

Of the 5 suprarenalectomized animals inoculated with the avian bacilli, all were found at autopsy to have extensive tuberculous lesions. In the previous experiment it was shown that only 50% of normal rats inoculated with the avian strain developed characteristic tuberculous lesions. It was, therefore, deemed unnecessary to use additional controls for this group.

It appears then that bilateral suprarenalectomy in the albino rat lowers the resistance so that subsequent inoculation with the bovine tubercle bacillus results in definite tissue reaction, with tubercle formation and caseation, resembling human tuberculosis. This does not occur in normal rats or in rats in restricted diet. Suprarenalectomy and, to a less extent, deficient diet increase the susceptibility of rats to the avian tubercle bacillus.

4649

Vitamin D in Tuberculosis.

BENJAMIN KRAMER, HAROLD G. GRAYZEL AND M. J. SHEAR.

From the Pediatric Research Laboratory, The Jewish Hospital of Brooklyn, New York.

In 1926 in the course of our vitamin and calcification studies[1, 2, 3] the question arose repeatedly as to whether vitamins play an important rôle in the etiology and treatment of tuberculosis. Ultra-violet light within recent years has been found of value in the treatment of intestinal tuberculosis; heliotherapy has been used with success in other forms of tuberculosis; calcification often is found in healed tuberculous lesions. These considerations suggested a possible linkage between tuberculosis and the vitamin D content of the diet.

In the older literature, reviewed by Wells, DeWitt and Long,[4] cod liver oil is mentioned as having value in tuberculosis. The bene-

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[1] Shear, M. J., and Kramer, B., *J. Biol. Chem.*, 1926, lxxi, 213.

[2] Kramer, B., Shear, M. J., and Shelling, D. H., *J. Biol. Chem.*, 1926, lxxi, 221.

[3] Shear, M. J., and Kramer, B., *J. Biol. Chem.*, 1928, lxxix, 105, 121, 125, 147, 161.

[4] Wells, H. G., DeWitt, L. M., and Long, E. R., "The Chemistry of Tuberculosis," Williams and Wilkins Co., Baltimore, 1923.

ficial results, sometimes obtained, may not have been due to the fat but to the vitamins present in the oil. The failures in the past may have resulted from the inability of the patient to tolerate the large amounts of fat which accompany the potent material in the oil. Or the patient may have been given cod liver oil devoid of vitamins.

In the present study only such materials were used as were shown to be antiricketically potent by biological assay. We prepared irradiated cholesterol and tested its potency on ricketic rats according to a previously described technique.[1, 2] We also used cod liver oil concentrate (Metz), obtained and tested as described in a preceding paper.[5] Also dried yeast powder (Fleischmann) was irradiated and biologically tested in an analogous fashion.

Subsequently, biologically assayed cod liver oil was added to the number of preparations studied. In the summer of 1926 we invited Dr. Bray and his staff, of the New York State Hospital for Incipient Tuberculosis at Ray Brook, to collaborate with us in that portion of the general vitamin-tuberculosis problem which pertained to human intestinal tuberculosis. They agreed to do the clinical testing of these materials at Ray Brook. They fed their patients daily, irradiated cholesterol, 2½ gm.; cod liver oil concentrate, 1 gm.; irradiated yeast, 10 gm. They[6] have so far reported to us that: orange or tomato juice aids in the retention of cod liver oil; the above mentioned agents, in conjunction with orange or tomato juice, are equally effective with the exception of irradiated cholesterol, which appears to be less effective than the others; 58 cases were treated, of which 50 received cod liver oil with tomato juice; the oral administration of these agents is at least as effective as artificial heliotherapy in the treatment of intestinal tuberculosis. The clinical details of this part of the work will be reported by Dr. McConkey, of Ray Brook.

In a separate series of cases we tested the efficacy of vitamin D unaccompanied by regular large additions of vitamins A and C. These clinical tests were carried out at the Neponsit Beach Hospital for Children.

Eighteen tuberculous children were divided into 2 groups of 9 each; one was the test group, the other the control. One child had tuberculosis of the skin, the others had active bone tuberculosis. All received a well-balanced diet. The treated children received daily 4 mg. irradiated ergosterol (Fleischmann) during the first 4 months and 7 mg. for the succeeding 8 months. (0.0001 mg. of the irradiated ergosterol per rat per day produced a continuous line in ricketic rats in 7 days.)

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[5] Kramer, B., Kramer, S. D., Shelling, D. H., and Shear, M. J., *J. Biol. Chem.*, 1927, lxxi, 699.

[6] Personal communications from Dr. Bray and Dr. Smith.

Physical examinations of the children were made periodically as well as blood pressure readings, urine analyses, roentgenograms and analyses for serum calcium and phosphorus. The results may be summarized as follows:

1. The daily addition of a large amount of vitamin D did not produce any detectable acceleration of the healing process.
2. Such doses of irradiated ergosterol produced no discernible deleterious effects. According to some observers, toxic symptoms, *e. g.:* anorexia, vomiting, pallor, loss of weight, hematuria, hyperphosphataemia and hypercalcemia, follow the administration of this agent. Our cases showed no such harmful effects.
3. The administration of irradiated ergosterol for 12 months produced no increase in either serum calcium or phosphorus.

4650

Identification of Blood Serum by Precipitin Reaction.*

HAROLD RECLUS WOLFE. (Introduced by T. C. Nelson.)

From the Department of Zoology, Rutgers University.

The precipitin reaction has been used extensively in medico-legal work for almost 30 years. Nuttall[1] used the precipitin tests in zoological relationships and Boyden[2] confirmed the work of Nuttall and stressed the principle of reciprocal relationships. The methods used in medico-legal work have been entirely of the "trial and error" type and no satisfactory method is known for identification of the individual bloods of a mixture, often an important item in criminal trials. Zoological relationships based on serological evidence can give a basis for a less cumbersome technique in medico-legal work.

In verifying the principle of reciprocal relationships and the per cent values of relationships the methods of Boyden were used. Two per cent antigen solutions of human, horse, sheep, beef, pig, cat and rat sera were made and the protein percentage determined. Antisera of all but the cat and rat sera were used. Two and often 3 readings, were made of each reaction and they agreed very well within themselves. A comparison of some of the relationships obtained with those of Boyden and of Nuttall showed a good agreement. The general correspondence of these results demonstrates the value of this biological method for the study of relationships.

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* The writer wishes to express his appreciation to Dr. A. A. Boyden of this University for his suggestions, aid and criticism of the problem investigated.

[1] Nuttall, G. H. F., Blood Immunity and Relationship, Cambridge University Press, 1904.

[2] Boyden, A. A., *Biol. Bull.*, 1926, vl, ...

The reciprocal values of the antisera employed in the preceding experiments are shown in Table I. Of the 18 reciprocal values shown, 13 are within the range of the error of the test (100% possible error). Three of the remaining 5 which show a greater divergence are anti-horse sera reactions, which have been consistently irregular in previous tests.

The work shows that in all cases the heterologous reactions were weaker that the homologous ones by definite per cents of the homologous titers. Hence in the identification of an unknown stain, if the protein content is determined, the first test can give a clue as to the possible origin of the blood by the percentage of the reaction. For example, if an anti-pig serum of known strength was used to identify a blood stain, a 100% reaction would indicate a homologous serum. On the other hand, if the reaction was only 5% of the homologous titer we would draw a tentative conclusion that the

TABLE I.
Reciprocal Values of Antisera.

Anti-serum	Antigen	% Relationships	Antiserum	Antigen	% Relationships
♀ D Beef	Sheep	37.5	Sheep ♀ H	Beef	37.5
	Horse	12.5	Horse ♀ F	”	12.5
	Pig	37.5	Pig ♀ C	”	9.47
	Human	4.67	Human ♀ G	”	6.25
♀ H Sheep	Pig	37.5	Pig ♀ C	Sheep	18.75
	Horse	18.75	Horse ♀ F	”	1.56
	Human	18.75	Human ♀ G	”	3.1
♀ F Horse	Pig	1.56	Pig ♀ C	Horse	9.47
	Human	7.56	Human ♀ G	”	12.5
♀ C Pig	Human	2.33	Human ♀ G	Pig	3.1
♀ B Pig	Beef	25.0	Beef ♀ D	Pig	37.5
	Horse	3.1	Horse ♀ F	”	1.56
	Human	3.1	Human ♀ G	”	3.1
	Sheep	18.75	Sheep ♀ F	”	37.5
♂ A	Horse	12.5	Horse ♀ F	Beef	12.5
	Sheep	50.0	Sheep ♀ H	”	37.5
	Human	12.5	Human ♀ G	”	12.5
	Pig	25.0	Pig ♀ B	”	25.0

stain was due either to the presence of human or cat serum. (This conclusion can be drawn from the relationship values previously obtained.) The next step would be to test for human or cat blood. The investigators using the "trial and error" method would (if the reaction was not 100%) proceed to test for beef, sheep, horse and other bloods until they got a positive test. Certainly this latter method is laborious and indirect.

In experimenting with the value of the precipitin test in the identification of mixtures of unknown bloods 3 methods were tried, only one being successful. The successful method was that of specific absorption. Various antisera were absorbed with heterologous serum and this took out the antibodies for the heterologous serum and left only specific antibodies. This absorbed antiserum would then identify only a homologous serum in a blood mixture. By using several different absorbed antisera the components of a mixture could be positively identified for the "Mammalian Reaction" of Nuttall did not occur in the dilutions of antigen employed. Two controls, besides the usual antiserum control, are necessary in performing these tests. One control contains the absorbed antiserum plus the heterologous antigen used for absorption and the other contains the absorbed antiserum plus the homologous serum. Practice in diagnosis included, first, tests of known mixtures and, secondly, unknown mixtures prepared by Dr. Boyden. The individual bloods of 4 unknowns were identified and the quantity of each was closely approximated, thus showing the trustworthiness of the technique employed.

4651

Dietary Requirements for Fertility and Lactation: XXI. Further Studies of the Role of Wheat Oil in Lactation.*

BARNETT SURE. (With the assistance of Alethea Beach.)

From the Laboratory of Agricultural Chemistry, University of Arkansas, Fayetteville.

In the early attempts to prevent sterility on a skimmed milk powder reproduction-deficient diet, the addition of wheat oil resulted not only in fertility, but also in excellent lactation when that oil constituted as much as 3% of the ration. The reduction of the concentration of the oil to 1% did not produce any significant change in fertility but was followed by a greater infant mortality. Similar results were obtained by the addition of various concentrations of corn oil and of cotton seed oil to the sterility-producing diet.[1] That phenomenon was used as one of the arguments that vitamin E may be composed of 2 fat-soluble vitamins, one essential for fertility, and another indispensable for lactation. It was stated, however, in previous communications that convincing evidence for such an hypothesis was still lacking.[1] The inability to secure evidence for the existence of a specific fat-soluble galactagogic vitamin on a dietary regime composed of purified food substances[2] stimulated a further attack on the problem. In an attempt to secure more conclusive information, Sherman and Muhlfeld's diet[3] (composed of whole wheat, 66.7; skimmed milk powder, 32; NaCl, 1.3) was adopted. This ration contains approximately 1.3% wheat oil as a source of vitamin E, furnished by the wheat,[4] and if vitamin E plays a specific rôle in lactation, it was anticipated that the further increase of wheat oil to the extent of 3% in the total diet, after vitamins A and D have been amply provided for, should produce an appreciable increase in the lactation efficiency index. Accordingly, the above diet was first supplemented with a brand of cod liver oil found in previous trial in this laboratory to be entirely deficient in vitamin E.[5] The cod liver oil was administered separately from the ration, in dosages of 0.1 cc., 0.2 cc., and 0.3 cc. daily to each animal. One to 2% wheat oil, and also one to 2% butter fat (the latter fat, being deficient in vitamin E,[6] served as a control) were added to the diet, which was supplemented by the varying amounts of cod liver oil, as a source of vitamins A and D. The optimum results in lactation were secured with the daily dosage of 0.3 cc. cod liver oil, as a supplement to the Sherman Muhlfeld diet. It also became apparent that, in the presence of daily allowance of 0.1 cc. cod liver oil, the addition of small amounts of butter fat and wheat oil supplemented that diet. Since I have already demonstrated[7] that wheat oil, in addition to being the most potent source of vitamin E, also contains appreciable amounts of vitamin A, it is quite possible that wheat oil supplemented the above diet by virtue of its vitamin A, and not by its vitamin E content. It is also quite possible that in previous studies, involving additions of cod liver oil to skimmed milk powder diets, there has been considerable destruction of vitamin A, which is suggestive from the work of Anderegg and Nelson[8] and from the studies of Mattill,[9] and that wheat oil acted partly as a protective mechanism against vitamin A oxidation[9] and partly as a vitamin A supplement, as indicated by the results of this investigation. That vitamin A is a limiting factor in the diet referred to from the standpoint of growth, reproduction and longevity has already been demonstrated by Sherman and MacLeod.[10]

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* Research paper No. 126, Journal Series, University of Arkansas.

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[1] Sure, B., *J. Biol. Chem.*, 1926, lxix, 29, 53.

[2] Sure, B., *Ibid.*, 1928, lxxvi, 659.

[3] Sherman, H. C., and Muhlfeld, M., *Ibid.*, 1922, liii, 41.

[4] Henry, W. A., and Morrison, F. B., Feeds and Feeding, 18th ed., 1913, 712.

[5] Sure, B., *J. Biol. Chem.*, 1927, lxxiv, 45.

[6] Sure, B., *Ibid.*, 1927, lxxiv, 71.

[7] Sure, B., *J. Agr. Res.*, 1928, xxxvii, 93.

4652

Phytopharmacological Reactions of Blood Serum from Leprosy, Tuberculosis and Syphilis.

DAVID I. MACHT.

From the Pharmacological Research Laboratory, Hynson, Westcott and Dunning, Baltimore, Maryland.

The author has called attention to the fact that by means of living plant-physiological test objects various toxins could be detected in the human blood which cannot be demonstrated by zoopharmacological methods. A marked toxicity was noted by Macht and Lubin in the blood of women at the beginning of the menstrual period.[1] Macht has demonstrated the presence of a toxin in the blood of pernicious anemia patients,[2] which led to useful clinical applications in respect to differential diagnosis and evaluation of therapeutic procedures.[3] The author, in collaboration with Pels, has shown also a toxin to be present in the blood serum and in the contents of the vesicles of patients suffering from pemphigus.[4] In the present communication, the results of an extensive study of a series of blood sera from patients suffering with tuberculosis, syphilis and leprosy, respectively, are reported.

A series of blood specimens from tuberculous patients were obtained, partly from the Tuberculosis Clinic of the Johns Hopkins

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[8] Anderegg, L. T., and Nelson, V. E., *J. Ind. and Eng. Chem.*, 1925, xvii, 451.

[9] Mattill, H. A., *J. Am. Med. Assn.*, 1927, xci, 1505.

[10] Sherman, H. C., and MacLeod, F. L., *J. Am. Chem. Soc.*, 1925, xlvii, 1658.

[1] Macht and Lubin, *J. Phar. Exp. Therap.*, 1924, xxii, 413.

[2] Macht, *J. Phar. Exp. Therap.*, 1926, xxix, 461.

[3] Macht and Anderson, *J. Phar. Exp. Therap.*, 1928, xxxiv, 365.

[4] Pels and Macht, *Arch. Dermatol. and Syphilol.*, 1929, xix, 640.

Hospital and partly from the Maryland Tuberculosis Sanatorium. A phytopharmacological examination of these sera in respect to their effect on the growth of *Lupinus albus* in Shive solution, according to the usual procedure employed by the author, revealed an average phytotoxic index of 78%, which is slightly higher than that of normal human blood serum.

A series of positive syphilitic sera, showing (+++) Wassermann reaction, obtained from the Wassermann Clinic of the Johns Hopkins Hospital, gave an average phytotoxic index for syphilis of 81%. This is definitely higher than the normal phytotoxic index for human blood serum.

The author has had an opportunity of studying a series of blood serum specimens from cases of leprosy. Some of these were obtained from the National Leprosarium, Carville, Louisiana, and others from the School of Tropical Medicine, Calcutta, India, through the courtesy of Prof. R. N. Chopra. An examination of these specimens revealed that the serum of leprosy is *markedly toxic,* the average phytotoxic index for 22 specimens examined being 47%.

A study of the effects of ultraviolet irradiation on leprosy serum in quartz tubes gave a further interesting finding. It was found that, whereas the serum of pernicious anemia is detoxified by irradiation with a quartz lamp, the serum of leprosy was not at all affected by such irradiation. This is not surprising in view of the fact that leprosy is most prevalent in tropical regions and the fact that it has been found that irradiation with ultraviolet rays does not cure the disease.[5] No definite effect was noted with irradiation of sera from tuberculosis and syphilis. Attention has already been called to the fact that in case of menstrual sera the effect of ultraviolet irradiation, if any, is to render the specimen more toxic.[6] The subjoined table shows the results obtained by the author in various clinical conditions. It will be noted that the readings obtained with

TABLE I.—*Comparative Table of Blood Sera.*

Kind of Blood Serum	Average of No. of Cases	Phytotoxic Index	Effect of Ultraviolet Irradiation
		%	
Normal human	100	72	No effect
Menstrual	50	51	More toxic
Pernicious anemia	48	44	Detoxification
Pemphigus	18	54	Variable
Leprosy	22	47	Not detoxified
Tuberculosis	50	78	No change
Syphilis	50	81	No change

[5] Macht, *J. P. I. Med. Assn.*, 1928, viii, 523.

[6] Macht, PROC. SOC. EXP. BIOL. AND MED., 1927, xxiv, 966.

leprosy serum showed such a marked toxicity, as compared with the less than normal toxicity of tuberculosis and syphilis, that the phytopharmacological examination may be utilized to great advantage in differentiating leprosy from the other two diseases, which may simulate it.

4653

Action of Predigested Corpus Luteum Extract on Excised Vas Deferens.

D. I. MACHT, A. E. STICKELS AND H. P. LEACH.

From the Pharmacological Research Laboratory, Hynson, Westcott and Dunning, Baltimore, Maryland.

Macht and Matsumoto[1] called attention to the peculiar sensitiveness of smooth muscle from the excised surviving vas deferens to extracts of corpus luteum. Such extracts produce a marked contraction of the vas deferens which is exceeded only by contractions produced by epinephrin. Extracts of numerous other glands, applied to the excised surviving vas deferens in the same doses, produced only slight contractions of its smooth muscle. This peculiar selective effect of corpus luteum extract on the vas deferens is not exhibited by the follicular hormone of the ovary but is specific for the hormone of the corpus luteum. For this reason, the authors suggested its employment in the evaluation of corpus luteum products, especially as clinical experience seemed to point to a parallelism between the therapeutic potency of corpus luteum products and their degree of activity as expressed by the degree of contraction of the vas deferens.[2]

More recently Macht, Stickels and Seckinger have found that the vas deferens reaction gave results which were parallel to the inhibitory effects of corpus luteum extracts on the oestrus cycle of the guinea pig as judged by a microscopic study of vaginal smears.[3]

Inasmuch as some hormones, as epinephrin, exert their characteristic effects only on injection, while others, like thyroid glands, for instance, produce their specific therapeutic effects on oral administration as well as after injection, it was considered worth while to investigate whether the effect of digestive juices will destroy the activity of the ovarian hormones or not. This was deemed especially desirable in view of a recent statement made by Novak on purely *a priori* speculative grounds, unsupported by any experimental data, that "there is much reason to believe that the active principle of the ovary is destroyed by the alimentary juices."[4] In the present investigation, a careful comparison between extracts of corpus luteum, prepared from the normal and predigested gland substance, was made in respect to its activity on the contractions of the vas deferens.

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[1] Macht and Matsumoto, PROC. SOC. EXP. BIOL. AND MED., 1919, xvi, 86.

[2] Macht and Matsumoto, *J. Urol.*, 1919, iii, 63.

[3] Macht, Stickels and Seckinger, *Am. J. Physiol.*, 1929, lxxxviii, 65.

Three kinds of corpus luteum extracts were studied. Extract No. 1 was an ordinary stock extract of corpus luteum, prepared by extraction of the dried gland substance with 60% alcohol and deproteinizing with lead subacetate and removing the excess of lead with alcoholic sulphuric acid. Such an extract is evaporated to a small volume, neutralized and then filtered and gives an aqueous solution with a pH 6.2. Extract No. 2 of corpus luteum was prepared by first extracting the dry gland with ether in a Sohxlet apparatus and then treating as in the preceding case. Extract No. 3 was prepared by first extracting dry corpus luteum substance with ether, then digesting 200 gm. in 600 cc. of N/12 hydrochloric acid, plus 1 gm. of pepsin (1:3,000) for 3 hours at 37°C.; then again, for one hour at 40°C.; and for a third time for one hour at 52°C. After digestion with pepsin, the substance was neutralized with sodium bicarbonate and 6 cc. of N/10 sodium hydroxide were added. Then the digestion was carried on further with the addition of 9 gm. of pancreatin, plus 3 gm. of purified ox bile, for one and a half hours at 40°C. After this second digestion, alcohol was added in sufficient quantity to make a 60% solution and the precipitate was filtered off. The solids were reextracted with alcohol and the combined filtrates were treated with lead subacetate, then filtered again. The excess of lead in the filtrate was removed as in extract No. 1 and, finally, an aqueous extract was obtained as in the case of the other preparations.

The corpus luteum extracts, mentioned above, were tested in regard to their activity on the isolated surviving vas deferens of the white rat suspended in oxygenated Locke solution at 38°C. The figures show the results obtained. In Fig. 1 is shown, first the effect of 1/10 cc. of adrenalin solution (1:1,000) added to the 50 cc. of Locke in the chamber (A). After the maximum contraction was obtained, the preparation was washed with warm Locke solution until it was writing again at its original level. One cc. of preparation No. 2 was then introduced into the chamber in the Locke solution, producing the contraction indicated (B). The preparation was then washed again and treated with an equivalent amount of predi-

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[4] Novak, *J. Am. Med. Assn.*, 1928, xci, 607.

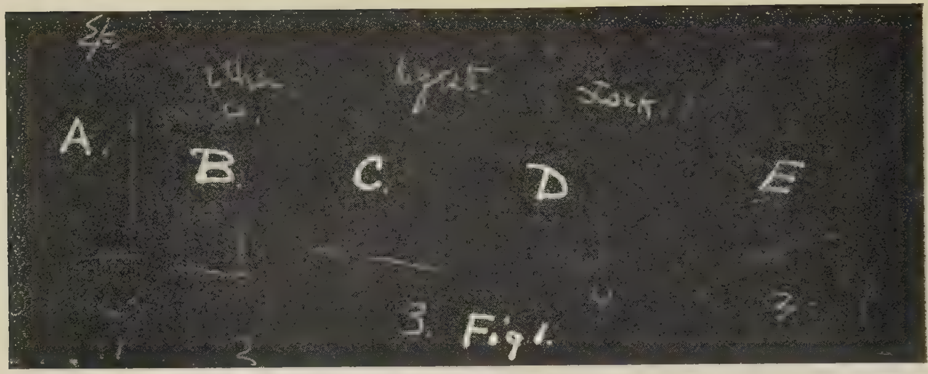

FIG. 1.

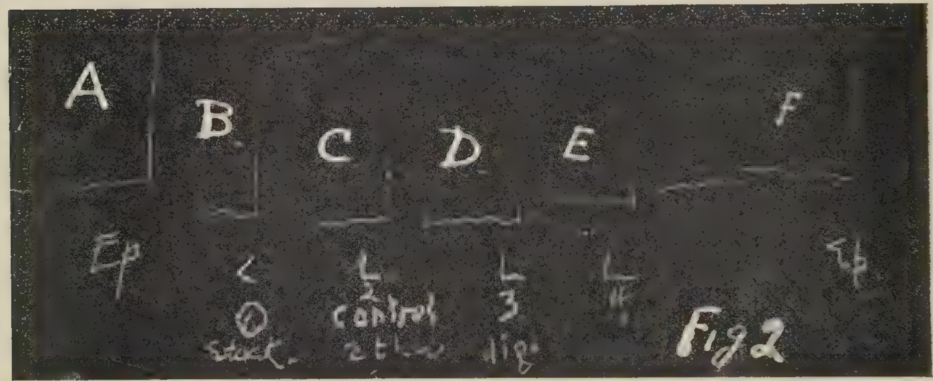

FIG. 2.

gested corpus luteum extract, again producing contraction, in this case slightly greater than the preceding (C). The preparation was washed again and treated for a third time with extract No. 1, again producing contraction (D). Finally, after another washing, the activity of the vas deferens was again tested with 1/10 cc. of adrenalin solution (E). In Fig. 2, the other vas deferens of the same animal was treated as follows: First, 1/10 cc. of epinephrine (A); second, 1 cc. of corpus luteum extract No. 1 (B); third, 1 cc. of corpus luteum No. 2 (C); fourth, predigested corpus luteum extract (D); fifth, a weaker corpus luteum extract preparation as in No. 1 (E); and sixth, 1/10 cc. of adrenalin (F).

The results obtained in the experiments described above, as well as in numerous others, indicate that even the most vigorous digestion of corpus luteum substance with pepsin, on the one hand, and with pancreatin and bile on the other, does not impair the activity of the corpus luteum extracts as far as their action on the vas deferens is concerned. Indeed, there seemed to be an indication that the predigested extracts were often more stimulating to the contractions than extracts obtained from undigested gland substance.

4654

On the Transmissibility of the Leucemia of Fowls.*

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Among approximately 190 fowls received between November and May from a poultry dealer for postmortem examination, 4 birds had a neoplasm and 2 others exhibited the pathological changes commonly termed leucemia of the fowl. One of these, a white Leghorn hen, showed on gross examination moderately enlarged, grayish kidneys and heart and on microscopic examination, an extensive diffuse infiltration of the kidney, heart, and thyroid gland, and a nodular infiltration of the liver with mostly mononuclear cells of medium size. The character of these cells could not be determined with certainty; the numerous transitional cells to red cells suggest that they may be hemoglobin-free precursors of erythrocytes. The number of white cells in the blood stream, as seen in sections, was not increased.

Emulsions of organs of this chicken have been injected (Passage I) into the veins of 4 white Leghorn and 3 barred Rock chickens. In about two and a half months one of the injected barred Rock chickens developed a severe anemia. There were many polychromatophile erythrocytes, numerous large lymphocyte-like cells in the blood stream and transitional forms between them. On microscopic examination the capillaries of several organs, particularly those of the liver and kidney, were found to be studded with such cells, which, in all probability, are progenitors of erythrocytes. This picture corresponds to what was called by Ellerman[1] "leucostasis" and the disease may be termed with him erythroleucosis.

The blood of this bird was injected (passage II) into the vein of 6 chickens, of which one developed in about 8 weeks a disease identical with that of the donor. Two other birds, one in about 6 and one in 11 weeks, showed an enormous increase of the white blood cells in the blood stream. The blood counts of the latter made with Toisson solution as diluting liquid were 530,000 to 670,000 white, and 1,550,000 to 1,800,000 red blood cells per cubic mm. The figures for hemoglobin were 35 to 38 (Sahli). The main type of cells in the blood smears stained with Wright solution alone or in combination with Giemsa stain were large mononuclear, poikilonuclear cells and myelocytes. In vital staining the majority of the mono- and poikilonuclear cells resembled myelocytes, the only marked difference being the absence of granules; there were many transitions between these cells, supporting the view of their myeloid origin (Ellerman). The most striking feature of the microscopic picture of the organs is the immense number of large mononuclear cells in the blood vessels and a widespread infiltration of the parenchymatous tissue with similar cells. These two cases may be termed, with Ellerman, myeloid leucosis. The 4 other birds of this series injected with organ emulsions of the same bird showed no symptoms of leucemia.

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* This investigation has been supported by a Fund for the Study of Leucemia and Related Diseases.

[1] Ellerman, W., The Leucosis of the Fowls and Leucaemia Problems, English edition, London, 1921.

Two further passages are now being followed. Of 12 birds injected (passage III) about 10 weeks ago with blood or with organ emulsions of one of the fowls afflicted with myeloid leucemia, one developed myeloid leucemia and another erythroleucemia, the findings being similar to those described above.

The criticism that the transmission experiments of Ellerman and others were carried out without any control (Pappenheimer[2]) cannot be applied to the present work. The occurrence of 3 cases of myeloid leucemia and 3 cases of erythroleucemia in the above 3 passages involving 29 birds is well contrasted by the absence of these diseases, with one exception, among approximately 200 birds used mainly in experiments directed toward the transmission of lymphomatous and other tumors and the production of leucemia. One chicken, which was injected daily with benzol mixed with olive oil, showed after the 12 subcutaneous injections a severe anaemia similar to those described above. A few of the numerous immature cells of the erythrocytic series in the blood stream were in mitotic division. The interpretation of this case requires further work.

Our findings are in accord with those of Schmeisser,[3] who observed in an experimental series involving 105 birds 22 cases of leucemia and anemia; he does not report, however, on the possibility of a clear-cut separation of the anemias from the myeloid leucemias. Ellerman's claim that the same transmissible agent may cause myeloid as well as lymphoid leucemia is not well supported. In 5 of his 8 transmission experiments (A, B, C, F, G) of which the first 3 involve 116 birds, no such change of type was observed. Among the passages of strain D, involving 21 birds, and strain H, involving 122 birds, one case of lymphoid leucosis was observed in each strain. A transformation in the reverse direction is based on the occurrence of one case of mild myeloid leucemia and another case of multiple myeloid tumors in strain E involving about 80 birds. These data are of little significance if one considers the frequent spontaneous occurrence of leucemia, particularly the lymphoid variety, among the birds of certain flocks. Thus Hennepe finds that 7% of the total deaths are caused by leucemia and 1.8% by tumors. Hennepe's (Holland) figures are higher than any other report available from the literature. Kerr finds that the incidence of mortality due to neoplasm is 9.8%; Schneider finds 2-3% of deaths are caused by tumors, and it is commonly accepted that lymphomatous tumors constitute the most common neoplastic disease of the fowl. Thus Tyzzer and Ordway found 7 among 9, and Feldman 11 among 19 tumors composed of lymphocyte-like cells. Mathews finds myeloid neoplasms in 0.5-1.3% of the birds examined post mortem.

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[2] Pappenheimer, A. M., Dunn, L. C., Cone, V., *Storrs Agr. Exp. Sta. Bull.*, 1926, No. 143.

[3] Schmeisser, H. C., *J. Exp. Med.*, 1915, xxii, 820.

In attempts to obtain transmissible strains of leucemia and lymphoma a large number of birds have been injected with organ emulsions of fowls 1-2 days after death of the bird or with still older frozen and defrosted material. The absence of leucemia among the injected birds contradicts the view of Schridde and McGowan[4] that in the fowl leucemia may be produced by the mechanical stimulation of injected organ emulsions.

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[4] McGowan, J. P., Pernicious Anaemia and Leucaemia and Aplastic Anaemia, New York, 1927.

4655

Amount of Ultra-violet Radiation Necessary to Cure Rickets.

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It has been amply proved that ultra-violet radiations have value in the cure and prevention of disease. Wave lengths of therapeutic value may vary for different diseases, but for rickets the band having a high antirachitic potency is a comparatively narrow one centering around line 2968 A. U.

The depth of penetration of the "vital rays" into the skin is perhaps not greater than 1½ mm., 95% of the rays being absorbed by the more superficial layers. Some of them do not penetrate the corneum. However, the most superficial blood vessels and nerves of the skin are within 0.2 of a mm. of the surface and are no doubt reached by many of the rays, such as the shortest received from the sun (2900 A. U.).

Rickets, a disease characterized by low blood calcium, lack of bone formation, low metabolism, retarded development, and weakness, responds so promptly and with such uniformity to irradiations from a source of known strength, that results can be predicted with almost the exactness of a chemical reaction.

In experimental and clinical work it has been the usual practice, in the past, to give much longer irradiations than are necessary. After four years experimenting with the problems of the minimum dose of ultra-violet rays necessary for satisfactory results in cure of rickets in chickens, we felt justified in making the following conclusions:

There was a total of 459 animals distributed among 7 series and 44 groups.

1. At a lamp floor distance of 22½ inches, one minute daily irradiation will bring about 95% cure in 28 days. It takes approximately 28 days to cure a severe form of the disease in chickens, no matter how much the irradiation is increased. At 30 inches about double this length of exposure is needed to bring about the same result. This corresponds to the law which states that the intensity is inversely proportional to the square of the distance.

When the total exposure was 37½ minutes, the chickens recovered about as rapidly if the treatments were given weekly instead of daily.

2. The benefit received from irradiation is proportional to the

unfeathered area exposed. Female chickens between 4 and 10 weeks of age require about double the irradiation needed for male birds of the same age. This is because the combs of the females are still small and rudimentary. Those of the males have usually begun to develop at 4 to 5 weeks, and have good circulation. The greater improvement of the males, when short exposures are given, is due to the greater uncovered area of their bodies and not to a sex difference.

The feathers of chickens prevent the rays from reaching the body.

Exposure of the unfeathered parts of the legs to ultra-violet rays, for 5 minutes daily, caused as rapid recovery as "total irradiation" for the same length of time.

4656

Experiments on Pleural Permeability.

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In connection with an extensive study on pleural effusions, it became necessary to collect data on the permeability of the normal pleura. Experimental data from the literature indicate a high degree of permeability; and Hamburger[1] in particular states that intraperitoneally or intrapericardially injected fluids are quickly changed to isotonicity with the blood serum and are then promptly resorbed. But we did not find any quantitative data which would answer our problem.

Several series of experiments on rabbits were performed; they all reveal, in accordance with previous workers, a very high degree of permeability of the pleural membrane. Two representative series of experiments will suffice to show the ready exchange which takes place between pleural cavity and blood.

In the first experiments the right pleural cavity of 9 rabbits was injected with 10 cc. of sterile, distilled water. The animals were killed 5, 7½, 15, 30, 45, 60, 120, 180, and 240 minutes after the injection. All fluid in the right pleural cavity was collected immediately, measured and examined (depending on a sufficient amount) for chlorides, glucose, sodium, calcium, nonprotein nitrogen, total serum nitrogen, magnesium and potassium. Immediately before death, a sufficient amount of blood was obtained from the carotid artery of each animal for the performance of the same determinations. Since the actual amounts of the various constituents showed considerable variation in the different animals, we have calculated the ratio of the concentration in the pleural fluid to the concentration in the blood, for each constituent determined. The ratios increase rapidly throughout the period of observation, although at a different rate for the various constituents. That the permeability of the pleura for a substance is not simply a function of the size of the molecule is indicated by the fact that the concentration of glucose in the pleural fluid reaches 100% of the blood value more quickly than does that of some inorganic components. Equality between pleural fluid and blood is established for chloride within 30 minutes, for glucose in 50 minutes, for potassium in 3 hours. Sodium, calcium and magnesium reach 70 to 80% of the blood plasma values within 1 hour. Nonprotein nitrogen is 50% after 30 minutes, while the total nitrogen, determined only during the first 15 minutes, is 15% of the serum nitrogen. The total amount of fluid decreases to 3.5 cc. during the first half hour; at the end of 6 hours the pleural cavity is dry. It is thus shown that, although the tendency to osmotic equilibrium is obvious throughout the course of the experiment, resorption begins before isotonicity is reached.

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[1] Hamburger, H. J., *Arch. f. Anat. u. Physiol., Physiol. Abtlg.*, 1895, 281.

In a second series of experiments, rabbits were injected in the same way as above but with hypertonic solution containing potassium chloride, glucose, and calcium lactate, the analysis of which revealed the following composition: potassium 432 mg., glucose 472 mg., calcium 44.5 mg., chlorides 357 mg., per 100 cc. Within the first hour after injection, the glucose concentration in the artificial pleural fluid is equal to that of the blood; the calcium content decreases during the same period from 350 to 250% of the blood value; potassium decreases to equality with the blood plasma content. At the same time, sodium and nitrogenous substances enter the pleural space, to the extent of 50 and 4% of the blood concentration, respectively. The resorption of the fluid is somewhat less rapid during the first half hour, but more rapid thereafter, than in the animals which had received distilled water.

Extensive chemical data upon spontaneous pleural effusions in humans show that the composition of these fluids is qualitatively and quantitatively in no essential way different from that of the blood plasma. This fact indicates clearly, in combination with the high degree of permeability demonstrated experimentally, that the failure of resorption in disease is not a function of the chemical composition of the effusions, but that it must be explained by the altered permeability of the pleural membrane.